FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **217039US0XPCT**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/926799

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/04095	**22 JUNE 2000**	**22 JUNE 1999**

TITLE OF INVENTION

SRSV DETECTION KIT

APPLICANT(S) FOR DO/EO/US

Naokazu TAKEDA, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority / PCT/IB/304 / PCT/IB/308
PTO-1449 / Drawings (6 sheets)

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR) 09/926799	INTERNATIONAL APPLICATION NO. PCT/JP00/04095	ATTORNEY'S DOCKET NUMBER 217039US0XPCT

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$890.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	14 - 20 =	0	x $18.00	**$0.00**
Independent claims	7 - 3 =	4	x $84.00	**$336.00**
Multiple Dependent Claims **(check if applicable)**.			☒	**$280.00**
TOTAL OF ABOVE CALCULATIONS =				**$1,506.00**
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**
SUBTOTAL =				**$1,506.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**
TOTAL NATIONAL FEE =				**$1,506.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**
TOTAL FEES ENCLOSED =				**$1,506.00**
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$1,506.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

(703) 413-3000

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Dec. 20 2001
DATE

DESCRIPTION

SRSV DETECTION KIT

Technical Field

This invention relates to a kit for detecting and distinguishing one or more small round structure viruses (hereinafter called "SRSVs") in a specimen.

Background Art

SRSVs are a group of causative viruses of human viral gastroenteritis, the discovery of the first one of which goes back to 1972. They are known to cause infantile acute gastroenteritis and also outbreaks of food poisoning or the like among adults and preschool or elementary school children. Due to the inability to proliferate these SRSVs by cell culture and the lack of animal models capable of exhibiting sensitivity thereto, SRSV antigens and anti-SRSV antibodies are hardly available, resulting in a delay in the development of immunoserologic methods for the detection of the viruses.

Under such circumstances, it was succeeded to clone the gene of the Norwalk virus, an SRSV, in 1993, leading to the determination of the base sequence of its complete genomes [JP(PCT) 6-506823 A]. Subsequently, PCR methods which are useful to amplify a part of an RNA polymerase region were developed, and 14 SRSV-related viruses have been found to date. As a result

of analyses of about 120 amino acids in these RNA polymerase regions, SRSVs are considered to be roughly differentiated into two genogroups, that is, Genogroup I including the Norwalk virus strain as a prototype and Genogroup II including the Snow Mountain virus strain as a prototype.

As genetic analyses of SRSV-related viruses proceeded, it came to knowledge that substantial diversity exists even in the same genogroup. As a matter of fact, it was found that with an RT-PCR method making use of primers for the genes of the Norwalk virus and Snow Mountain virus strains as the prototypes of the respective genogroups, every SRSV is not detectable and also that it is very difficult to design primers or set RT-PCT conditions for achieving efficient amplification of SRSVs.

In the meantime, antigens were prepared against some of the viruses, such as the Norwalk virus strain and the Snow Mountain strain, by genetic expression, antibodies were obtained, and ELISA-dependent SRSV detection methods making use of such antibodies were also developed. It was, however, still impossible to detect every gastroenteritis-causing SRSV due to the diversity of the SRSVs.

In Japan, on the other hand, SRSVs were designated in 1997 to be causative factors of food poisoning as defined in the Food Sanitation Act so that, if SRSV food poisoning breaks out, determination of its infection route is required. There is accordingly a desire for a method which easily and surely detects

and identifies SRSVs in infected subjects' feces or foods.

Disclosure of the Invention

Accordingly, an object of the present invention is to provide a kit which can easily detect from a specimen an SRSV-related virus known to date and can surely discriminate its serotype and genogroup.

With the foregoing circumstances in view, the present inventors have proceeded with an genetic and immunological investigation on SRSV-related viruses. As a result, it has been found that combined use of antibodies obtained from 11 SRSV-related virus peptides, including newly-found novel virus peptides, can detect most SRSVs in specimens and can surely discriminate the serotypes and genogroups of the SRSVs, leading to the completion of the present invention.

Specifically, the present invention provides an SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

The present invention also provides an SRSV detection kit for discriminating SRSVs in genogroup, said SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (d), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Further, the present invention also provides an SRSV detection kit for discriminating genogroup of SRSVs, said SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide

groups (e) to (k), respectively:

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

Furthermore, the present invention also provides SRSV-related virus strain genes having base sequences

represented by SEQ ID NOS: 15, 20, 21 and 22 or base sequences similar to the first-mentioned base sequences, respectively, except for deletion, replacement or addition of one to several bases of said first-mentioned base sequences.

Brief Description of the Drawings

FIG. 1 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Seto 124/1989/JP strain.

FIG. 2 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Funabashi 258/1996/JP strain.

FIG. 3 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chiba 407/1987/JP strain.

FIG. 4 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Narita 104/1997/JP strain.

FIG. 5 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Sanbu 809/1998/JP strain.

FIG. 6 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Ichikawa 754/1998/JP strain.

FIG. 7 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Chitta 1876/1996/JP strain.

FIG. 8 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Kashiwa 47/1997/JP strain.

FIG. 9 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Mie 7k/1994/JP strain.

FIG. 10 is an electron micrograph (x 100,00) of virus-like

particles derived from the Hu/NLV/Kashiwa 645/1999/JP strain.

FIG. 11 is an electron micrograph (x 100,00) of virus-like particles derived from the Hu/NLV/Osaka 10-25/1999/JP strain.

Best Modes for Carrying Out the Invention

1. SRSV-related viruses

The SRSV detection kit according to the present invention is characterized by the use of the antibodies against SRSV-related virus constituting peptides having the 11 specific amino acid sequences or at least 80% of homologies with the amino acid sequences in the groups (a) to (k). Of these, the peptides belonging to the group (d), the group (i), the group (j) and the group (k) are novel peptides different from any SRSV-related viruses registered with the GeneBank to date (Table 1, which will be described subsequently herein). Owing to the incorporation of the 11 antibodies, including antibodies against these novel peptides, into the kit, SRSV-related viruses can be detected without omission.

The SRSV-related virus constituting peptides useful in the present invention embrace their mutants in each of which one or more amino acids have been deleted from, replaced in or added to its corresponding amino acid sequence; and also their mutants in each of which one or several bases have been deleted from, replaced in or added to a base sequence encoding its corresponding amino acid sequence.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 1 in the group (a) is a virus constituting peptide of the Hu/NLV/Kashiwa 645/1999/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Desert Shield/90/SA strain (GeneBank Accession No. U04469).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 2 in the group (b) is a virus constituting peptide of the Hu/NLV/Seto 124/1989/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the KY-89/89J strain (GeneBank Accession No. L23828) and the Norwalk/68/US strain (GeneBank Accession No. M876611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 3 in the group (c) is a virus constituting peptide of the Hu/NLV/Funabashi 258/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Southampton/91/UK strain (GeneBank Accession No. L07418).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 4 in the group (d) is a virus constituting peptide of the Hu/NLV/Chiba 407/1987/JP strain

obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 4 has less than 75% of homology in structural gene (SEQ ID NO: 15) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 5 in the group (e) is a virus constituting peptide of the Hu/NLV/Narita 104/1997/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Bristol/93/UK strain (GeneBank Accession No. X76716), the Lordsdale/93/UK strain (GeneBank Accession No. X86557), and the Camberwell/94/AU strain (GeneBank Accession No. U46500).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 6 in the group (f) is a virus constituting peptide of the Hu/NLV/Sanbu 809/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Mexico/89/MEX strain (GeneBank Accession No. U22498), the Auckland strain (GeneBank Accession No. U460391), the Toronto/77/CA strain (GeneBank Accession No. U02030), and the

OTH-25/89/J strain (GeneBank Accession No. L23830).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 7 in the group (g) is a virus constituting peptide of the Hu/NLV/Ichikawa 754/1998/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include those derived from the Snow Mountain/76/US strain (GeneBank Accession No. U70059) and the Melksham/89/UK strain (GeneBank Accession No. X81879).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 8 in the group (h) is a virus constituting peptide of the Hu/NLV/Chitta 1876/1996/JP strain obtained from feces of an SRSV infected patient in Japan, whereas examples of the peptides each having at least 80% of homology with the amino acid sequence include one derived from the Hawaii/71/US strain (GeneBank Accession No. U07611).

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 9 in the group (i) is a virus constituting peptide of the Hu/NLV/Kashiwa 47/1997/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 9 has less than 75% of homology in structural gene (SEQ ID NO: 20) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence

not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 10 in the group (j) is a virus constituting peptide of the Hu/NLV/Mie 7k/1994/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 10 has less than 70% of homology in structural gene (SEQ ID NO: 21) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Illustrative of the peptide having the amino acid sequence represented by the SEQ ID NO: 11 in the group (k) is a virus constituting peptide of the Hu/NLV/Osaka 10-25/1999/JP strain obtained from feces of an SRSV infected patient in Japan.

The peptide having the amino acid sequence represented by SEQ ID NO: 11 has less than 70% of homology in structural gene (SEQ ID NO: 22) with any one of the SRSV-related virus strains (Table 1, which will be described subsequently herein) registered with the GeneBank to date, and is a peptide having a novel sequence not reported to date.

Table 1

Virus strain	GeneBank Accession No.
Desert Shield/90/SA	U04469
Norwalk/68/US	M876611
KY-89/89J	L23828
OTH-25/89/J	L23830
Southampton/91/UK	L07418
Lordsdale/93/UK	X86557
Bristol/93/UK	X76716
Camberwell/94/AU	U46500
Toronto/77/CA	U02030
Mexico/89/MEX	U22498
Snow Mountain/76/US	U70059
Melksham/89/UK	X81879
Auckland	U460391
Hawaii/71/US	U07611

The SRSV-related virus constituting peptides in these groups (a) to (k) embrace, in addition to the above-described peptides, partial peptides each of which contains a specific amino acid sequence in its corresponding peptide and has antigenecity equivalent to the corresponding peptide.

According to a homological analysis of about 120 amino acids of RNA polymerase regions of the SRSV-related virus constituting peptides, these SRSV-related virus constituting peptides can be classified into two genogroups. Described

specifically, they can be classified into Type I to which the peptides in the groups (a) to (d) belong and Type II to which the peptides in the groups (e) to (k) belong.

2. Cloning of the SRSV-related virus constituting genes

From feces of an SRSV infected patient, viral RNA is extracted using the cetyltrimethylammonium bromide (CTAB) method or the like, cDNA was formed by an oligo-dT primer and a reverse transcriptase, and using the cDNA and primers capable of amplifying structural gene regions of the individual SRSV-associate viruses, PCR was conducted to amplify structural gene fragments.

Such a structural gene fragment is inserted in a plasmid by once conducting TA cloning with an *E. coli* cloning vector.

As a cloning vector usable here, it is possible to use a known cloning vector such as a vector derived from a plasmid obtained using as host procaryotic cells represented by *E. coli* or from a bacteriophage represented by ë phage, and appropriately combined use of a cloning vector and its host cell is desired. Specific examples of the cloning vector include pBR322, pUC19 and pCRII. The insertion of the DNA can be conducted by a method known *per se* in the art, and upon formation of such a vector, use of *E. coli* cells is desired as they permit easy genetic manipulation.

3. Expression of structural gene and creation of virus-like particles.

By having fragments of the above-obtained individual virus constituting genes in the groups (a) to (k) expressed with a suitable expression system or by using virus-like particles created from the virus constituting peptides in a genetic engineering manner, antibodies against the respective viruses can be obtained. A description will hereinafter be made about an expression when *E. coli* is used and also about the creation of virus-like particles.

(1) Expression by *E. coli*

Plasmids with the structural gene regions of the respective SRSV-related viruses incorporated therein, respectively, are each digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in pGEX (GST fusion protein expression vector; product of Pharmacia AB), pTrc99A (*E. coli* expression vector; product of Pharmacia AB), pTrxFus (thioredoxin fusion protein expression vector; product of Invitrogen Corporation), pET (expression vector making use of pT7RNA promoter; product of Novagen Inc.), a maltose binding protein expression vector, or a â galactosidase fusion protein expression vector. At this time, the structural gene region to be incorporated can be of the complete length or can be a partial region, with a partial region containing at least one antigen epitope of an SRSV being preferred. Gene expression vectors with the structural gene regions incorporated therein

as described above are transformed by an *E coli* strain suited for gene expression, for example, the BL21 strain, the DH10B strain, the JM109 strain or the XL1-Blue strain. Expression of the gene can be conducted by culturing the thus-obtained transformants in a general liquid culture medium, for example, L-broth. It is preferred for the expression to add a gene expression promoter, for example, IPTG or, when a PL promoter is used, to apply a heat shock.

Purification of a peptide so expressed can be conducted following a general purification method for expressed protein, which makes use of *E coli*. If the expressed protein is in a dissolved form, for example, its purification can be conducted by affinity chromatography making use of a GST column or a column for maltose binding proteins. If the expressed protein is in an insoluble form, its purification can be achieved by conducting affinity chromatography making use of a Ni chelate.

(2) Creation of SRSV virus-like particles

A plasmid with a structural gene region of an SRSV-related virus incorporated therein is digested with a restriction endonuclease which does not cleave the structural gene region. Then, the structural gene region is collected and incorporated, for example, in a baculovirus transfer vector such as pVL1393. The transfer vector and a linear baculovirus DNA, from which a gene region essential for proliferation has been deleted, are subjected to transfection in insect cells such that homologous

recombination is induced to form the target recombinant baculovirus.

By infecting the thus-obtained recombinant baculovirus to insect cells such as Sf9 cells or Tn5 cells and incubating the infected insect cells under adequate growth conditions in a manner known *per se* in the art, the structural protein of the SRSV is expressed. By allowing the structural protein to undergo self-assembly, virus-like particles can be produced. Use of a biochemical purification method, for example, centrifugation makes it possible to isolate and purify the virus-like particles. Whether or not such virus-like particles have been formed can be confirmed by subjecting the self-assembled product to negative staining with uranyl acetate and examining the stained self-assembled product by an electron microscope.

The virus-like particles obtained as described above do not have infectiveness as they do not contain any gene internally. Nonetheless, they have antigenecity equivalent to virus particles because they structurally have substantially the same shape as virus particles.

4. Acquisition of antibodies against SRSV-related viruses

By immunizing an animal with the thus-obtained virus constituting peptide or virus-like particles, an anti-SRSV-related virus antibody can be prepared. Incidentally, such an antibody can be either a monoclonal antibody or a polyclonal antibody.

Preparation of an immune antibody by making use of virus-like particles can be conducted, for example, as will be described next. In a manner known *per se* in the art, a rabbit is immunized with virus-like particles of one of the SRSV-related viruses, and from separated serum, an IgG antibody (anti-SRSV antibody) against the virus-like particles can be obtained. For the separation and isolation of the antibody, a method such as DEAE Sepharose chromatography can be used.

Using the 11 types of virus-like particles of the groups (a) to (k) obtained as described above and their corresponding anti-SRSV antibodies, their cross reactivities were measured. As will be shown below in Table 2, absolutely no cross-reactivity was exhibited between the individual SRSV-related viruses. According to the SRSV detection method of the present invention, it is therefore possible to concurrently discriminate the serotypes of 11 types of SRSVs. This also indicates the possibility of discriminating Genogroup I Genogroup II from each other at the same time.

5. Detection of SRSV-related viruses

For the detection of one or more SRSVs in a specimen by the individual anti-SRSV antibodies obtained as described, conventionally employed immunoassays making use of antigen-antibody reactions, for example, radioimmunoassay by the sandwich technique, enzyme-linked immunosorbent assay (ELISA) and the like can be used, with ELISA being particularly

preferred. Described specifically, the 11 types of anti-SRSV antibodies are separately poured into a microplate to prepare an SRSV screening plate. A dilution of a fecal emulsion, which has been prepared from feces of an SRSV infected patient, is added to the wells of the plate, and is then allowed to react. Peroxidase (POD) labeled anti-SRSV antibodies of the respective viruses are thereafter added and reacted. After a substrate solution (TMB containing hydrogen peroxide) is added and reacted, 0.6 N sulfuric acid is added to stop the reactions. By measuring the absorbance (450 nm/630 nm) of each well by an ELISA autoreader, the SRSV or SRSVs can be detected.

When it is desired to conduct only the detection of one or more SRSVs in a specimen, a detection kit can be prepared by using a microplate with all the 11 types of anti-SRSV antibodies mixed and immobilized thereon. To also discriminate even the serotypes of the one or more SRSVs, a detection kit can be prepared by using microplates with all the 11 types of anti-SRSV antibodies immobilized separately thereon.

Further, the discrimination of the genogroups is feasible by a kit making use of a microplate with antibodies against the peptides in the groups (a) to (d) mixed and immobilized thereon (Type I plate) or a microplate with antibodies against the peptides in the groups (e) to (k) mixed and immobilized thereon (Type II plate).

Moreover, immobilization of the individual anti-SRSV

antibodies useful in the present invention with a carrier such as a latex or magnetic beads makes it possible to surely capture one or more SRSV-related viruses in a specimen. The carrier with one or more SRSV-associate viruses captured thereon can be recovered by centrifugation in the case of the latex or by magnet in the case of the magnetic beads. Subsequent to the recovery, virus RNAs can be extracted and used.

Examples

The SRSV detection kits according to the present invention will hereinafter be specifically described based on Examples.

Example 1 Cloning of structural genes of SRSV-related viruses

(1) Synthesis of cDNA

PBS (9 mL) and "Daiflon" (1 mL) were added to feces (0.5 to 1.0 g) of an SRSV patient, followed by homogenization. The homogenate was then centrifuged at 3,000 rpm for 20 minutes, and the supernatant was collected as a 10% fecal emulsion.

Using a 1-mL aliquot of the fecal emulsion, RNA of the SRSV was extracted by the cetyltrimethylammonium bromide (CTAB) method, and the RNA was eventually suspended in a 0.1% diethyl pyrocarbonate solution (30 iL). Using the suspension, cDNA was prepared by a reverse transcriptase derived from the Oligo-dT(12-18) primer and AMV (Avian Myeloblastosis Virus) (product of SEIKAGAKU CORPORATION).

(2) Isolation of structural gene regions

Using the cDNA prepared in (1) and primers for amplifying the structural gene regions shown below, PCR was conducted. Subsequent to the PCR, amplified structural gene fragments were separated by agarose gel electrophoresis, and were then recovered by using "SuprecTM-01" (TAKARA).

Hu/NLV/Kashiwa 645/1999/JP gene:G1/F2(SEQ ID NO: 23), Oligo-dT(33) (SEQ ID NO: 24)

Hu/NLV/Seto 124/1989/JP gene:G1/F2(SEQ ID NO: 23),G1/R0 (SEQ ID NO: 25)

Hu/NLV/Funabashi 258/1996/JP gene:G1/F2(SEQ ID NO: 23), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Chiba 407/1987/JP gene:D5(SEQ ID NO: 26), CV-U4(SEQ ID NO: 27)

Hu/NLV/Narita 104/1997/JP gene:97k104/F1(SEQ ID NO: 28), 97k104/R1(SEQ ID NO: 29)

Hu/NLV/Sanbu 809/1998/JP gene:G2/F3(SEQ ID NO: 30), MV-R1(SEQ ID NO: 31)

Hu/NLV/Ichikawa 754/1998/JP gene:G2/F3(SEQ ID NO: 30), SMV-R1(SEQ ID NO: 32)

Hu/NLV/Chitta 1876/1996/JP gene:G2/F3(SEQ ID NO: 30), G2/R0(SEQ ID NO: 33)

Hu/NLV/Kashiwa 47/1997/JP gene:97k104/F1(SEQ ID NO: 28), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Mie 7k/1994/JP gene:G2/F3(SEQ ID NO: 30), Oligo-dT(33)(SEQ ID NO: 24)

Hu/NLV/Osaka 10-25/1999/JP gene:GFCR7(SEQ ID NO: 34), Oligo-dT(33)(SEQ ID NO: 24)

(3) Cloning of structural genes

TA cloning of the recovered structural gene fragments to an *E. coli* cloning vector, pCRII(product of Invitrogen Corporation) was conducted. Obtained from these clones were plasmids with the structural genes of the viruses incorporated therein, pCRII/645, pCRII/124, pCRII/258, pCRII/Chiba, pCRII/104, pCRII/809, pCRII/754, pCRII/76, pCRII/47, pCRII/7k, and pCRII/10-25.

Example 2 Determination of base sequences

Determination of the base sequences of the structural genes of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain was conducted in the below-described manner.

Firstly, a primer (first primer) was set in the vicinity of the polyhedrin promoter of pVL1393 as a transfer vector, and by the dye termination method, a labeling reaction was conducted by using a "Cycle Sequencing Kit FS" (product of Perkin-Elmer

Corp.). The DNA concentration of the transfer vector employed was 0.4 ig/iL, whereas the concentration of the sequencing primer used was 3.2 pmol/iL. Subsequent to the reaction, the excess fluorescent pigment was eliminated using a centriprep spin column (manufactured by Perkin-Elmer Corp.). The reaction mixture was completely dried by a vacuum lyophilizer, and the lyophilizate was suspended in a special sample buffer (20 iL; product of Perkin-Elmer Corp.). Subsequent to stirring, the suspension was subjected to centrifugal precipitation. The precipitate was dried at 95℃ for 2 minutes. After quenching, it was analyzed by an autosequencer ("ABI Genetic Analyzer 310").

Using the base sequence determined by the first primer, a new sequencing primer (second primer) was set on the 3'-side of the base sequence. Using this second primer, a labeling reaction was conducted by a cyclic sequencing kit in a similar manner as mentioned above. Subsequent to the reaction, operation similar to that mentioned above was performed, and the base sequence was analyzed by the autosequencer. As has been described above, a sequencing primer was set on the 3' side of the base sequence determined in each cycle, and determination of the base sequence was conducted. By repeating this procedure, the base sequences from the 5'-ends to the 3'-ends of the 11 types of SRSV-related virus structural genes (SEQ ID NO: 12 to SEQ ID NO 22) were determined. Among these, the base sequences represented by SEQ ID NO: 15 (the Hu/NLV/Chiba 407/1987/JP

strain), SEQ ID NO: 20 (the Hu/NLV/Kashiwa 47/1997/JP strain), SEQ ID NO: 21 (the Hu/NLV/Mie 7k/1994/JP strain) and SEQ ID NO: 22 (the Hu/NLV/Osaka 10-25/1999/JP strain) were confirmed to be novel sequences not reported to date.

Example 3 Creation of recombinant baculovirus capable of yielding virus-like particles

(1) Construction of transfer vectors

The plasmids with the structural gene regions incorporated therein, which had been obtained in Example 1(3), were digested by a restriction endonuclease which does not cleave the structural gene regions. Subsequent to separation by agarose gel electrophoresis, the structural gene regions were recovered by "SuprecTM01" (TAKARA). The recovered gene fragments were incorporated in baculovirus transfer vectors pVL1393 (product of Invitrogen Corporation), which had been digested by the same restriction endonuclease, to prepare transfer vectors.

(2) Creation of recombinant baculoviruses

Baculovirus DNA (0.5 ig; "Baculo-Gold") and one of the transfer vectors (1 ig) obtained in (1) were dissolved in distilled water (8 iL). The resulting solution was mixed with a two-fold dilution of lipofectin (equivalent amount), and the thus-obtained mixture was left over at room temperature for 15 minutes. After Sf9 cells (1×10^5 cells) suspended in an insect cell culture medium, "Ex-cell 400", were adsorbed at 26.5℃ for 30 minutes in a plastic Petri dish (diameter: 3.5 cm), a mixture

of the transfer vector and "Baculo-Gold" was added dropwise to the cells, followed by incubation at 26.5℃. 24 Hours later, the culture medium was replaced by a "TC100" (product of GIBCO BRL Life Technologies; hereinafter referred to as "TC100") which contained 10% fetal bovine serum and 2% BTB (products of GIBCO BRL Life Technologies), and incubation was continued further.

(3) Purification of recombinant baculoviruses

After each recombinant baculovirus obtained in (2) was incubated for 5 days, the culture supernatant was diluted tenfold with an insect cell culture medium such as TC100. A 0.1-mL aliquot of the diluted supernatant was taken, and inoculated to 3 x 10^6 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME (low melting-point agarose) was overlayed, followed by incubation at 26.5℃. On the 4^{th} day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was further overlayed, followed by incubation at 26.5℃. On the following day, the formed plaques were scraped off with a microtip. and were suspended in TC100 culture medium.

(4) Production of recombinant baculovirus seeds and measurement of their infective potencies

Each suspension obtained in (3) was inoculated to 1 x 10^7 Sf9 cells. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 was added, followed by incubation at 26.5℃ for 3 to 4

days. The culture was centrifuged at 2,500 rpm for 10 minutes at 4℃, and the culture supernatant was collected. The collected culture supernatant was inoculated to 1 x 10^7 Sf9 cells. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 was added, followed by incubation at 26.5℃ for 3 to 4 days.

Next, the culture supernatant was inoculated to 3 x 10^7 Sf9 cells cultured in a plastic Petri dish of 3.5 cm in diameter. Subsequent to adsorption at 26.5℃ for 60 minutes, TC100 culture medium (2 mL) which contained 1% of Agarose ME (low melting-point agarose) was overlayed, followed by incubation at 26.5℃. On the 4^{th} day after the initiation of the incubation, TC100 (1 mL) which contained 0.005% of neutral red was then overlayed, followed by incubation at 26.5℃. On the following day, the formed plaques were measured to calculate the infective potency of the recombinant baculovirus. This was recorded as the infective potency of the recombinant baculovirus.

Example 4 Creation of virus-like particles

(1) Expression of structural proteins by using recombinant baculoviruses

Each recombinant baculovirus was infected at M.O.I.s (Multiplicities of infection) of 1 to 10 to Sf9 insect cells. Upon infection, a suspension of the recombinant baculovirus was added dropwise to the cells, and the recombinant baculovirus was subjected to adsorption for about 60 minutes or so with gentle shaking. After that, TC100 was added as an insect cell culture

medium, followed by incubation at 26.5℃ for 5 to 6 days.

(2) Identification of expressed proteins

The culture supernatant of each recombinant virus infection was periodically sampled. After having been resolved by SDS-PAGE, the protein was detected by Coomassie blue staining, and by an expected molecular weight, the validity of the expressed protein was confirmation. Further, subsequent to resolving the protein by SDS-PAGE, the protein was transferred onto a nitrocellulose membrane, and by the Western blotting technique, the expressed protein was then identified with a convalescent serum of the SRSV.

(3) Purification and recovery of virus-like particles

The recombinant baculovirus seeds were infected at M.O.I.s of from 1 to 10. Subsequent to adsorption for about 60 minutes, "Ex-cell 400" was added, followed by incubation at 26.5℃ for 3 days. A protease inhibitor, for example, pepstatin A or a leupeptin, was then added to the culture to a final concentration of 1 mM, followed by further incubation for 2 to 3 days.

Subsequent to the incubation, the culture was centrifuged at 2,500 rpm for 10 minutes at 4℃ to collect the culture supernatant. The collected culture was centrifuged at 10,000 rpm for 30 minutes to eliminate the recombinant baculovirus. The supernatant was centrifuged at 25,000 rpm for 4 hours on a "Beckmann SW28 Rotor" to have virus-like particles precipitated. Then, the centrifuge tube from which the

supernatant had been discarded was held upside down to complete eliminate the supernatant. After that, Grace buffer or PBS(-) (0.5 mL) with the protease inhibitor added therein was added to the centrifuge tube, and the centrifuge was allowed to stand overnight at 4℃.

After the standing, the virus-like particles were suspended in the protease-inhibitor-containing Grace buffer which had been added, and were recovered. To the recovered virus-like particles, protease-inhibitor-containing Grace buffer or PBS(-) with CsCl (3.8 g) added therein was added to give 13 mL. The resulting mixture was ultracentrifuged at 16℃ and 35,000 rpm for 24 to 48 hours. Subsequent to the ultracentrifugation, a pale band in which virus-like particles gathered was collected. After 5-fold dilution with protease-inhibitor-containing Grace buffer, the resultant suspension was ultracentrifuged at 45,000 rpm for 3 hours on a "Beckmann TL100.3 Rotor" to have the virus-like particles precipitated.

The precipitated virus-like particles were solubilized with Grace buffer or PBS(-) to which the protease inhibitor had been added. Protease-inhibitor-containing Grace buffer solutions which contained 10% to 50% of sucrose were prepared in a 4PA tube, into which the solubilized solution of the virus-like particles was overlayed, followed by sucrose density-gradient centrifugation at 35,000 rpm for 4 hours at

4℃. Subsequent to the centrifugation, a pale band of virus-like particles was collected as purified SRSV virus-like particles in a 1-mL syringe fitted with a 26G needle.

The purified SRSV virus-like particles was diluted with Grace buffer as needed, and the quantity of protein was measured by the Bradford method.

The purified SRSV virus-like particles were subjected to negative staining with uranyl acetate, and were then examined by an electron microscope to ascertain whether or not virus-like particles had been formed (FIGS. 2 to 12).

Example 5 Preparation of Immune Antibodies and Labeled by Use of Antibodies Virus-like Particles

(1) Preparation of immune antibodies against virus-like particles

A phosphate buffer (pH 7.2, 1 mL) - which contained the purified SRSV virus-like particles (500 ig) obtained from one of the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain - and the Freund's incomplete adjuvant (1 mL) were mixed, and then immunized to a New Zealand white rabbit

(3 kg) in a manner known *per se* in the art. Three weeks later, the rabbit was immunized further with a mixture of a phosphate buffer (pH 7.2, 1 mL), which contained the SRSV virus-like particles (0.25 ig), and the Freund's incomplete adjuvant (1 mL) (booster dose). Additional 3 weeks later, immunization was conducted as in the booster dose, and about 7 to 10 days after the additional booster dose, exsanguination was conducted, and the serum component was separated.

After the separated and purified serum was subjected to ammonium sulfate fractionation, the relevant fraction was dialyzed overnight at 4℃ against 50 mM Tris-HCl (pH 7.6). The inner dialyzate was then subjected to DEAE Sepharose chromatography which had been equilibrated with 50 mM Tris-HCl (pH 7.6). Under monitoring at an UV wavelength of 280 nm, an O.D. peak was collected to obtain an DEAE-purified IgG antibody (anti-SRSV antibody) against the virus-like particles.

(2) Preparation of labeled antibodies

Each anti-SRSV antibody was labeled with POD by an improved periodic acid technique ["Koso Men-eki Sokuteiho (Enzyme Immunoassay)", 2, 91, 1982]. Described specifically, POD was dissolved at 4 mg/mL in distilled water and 0.1 M sodium periodate (0.2 mL) was added, followed by a reaction at room temperature for about 20 minutes. The reaction mixture was then dialyzed overnight against 1 mM sodium acetate buffer (pH 4.0). Subsequent to the dialysis, 0.2 M sodium carbonate buffer (pH

9.5, 0.02 mL) was added to adjust the pH to 9.5, and at the same time, the anti-SRSV antibody (8 mg) was added.

After having been allowed to react at room temperature for 2 hours, 4 mg/mL sodium borohydroxide (0.1 mL) was added, followed by a reaction at 4℃ for about 2 hours. After the reaction, gel filtration was conducted with "Sephacryl S-200" while using 10 mM phosphate buffer. Under monitoring at an UV wavelength of 280 nm, a POD-labeled anti-SRSV antibody fraction was collected.

(3) Preparation of a solid-phase anti-SRSV antibody microplate

The anti-SRSV antibodies were separately diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL and then poured at 100 iL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was then allowed to stand overnight 4℃. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 iL/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight 4℃ to obtain a solid-phase anti-SRSV antibody microplate.

Example 6 Cross-reactivity

(1) Antigen detection ELISA

The purified SRSV virus-like particles of each group were diluted to 4 ng/mL to 0.04 ng/mL with a solution containing bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.2% and 0.05%, respectively, in a buffer (10 mM PBS, pH 7.2).

Then, the diluted emulsions of the virus-like particles (VLPs) were each added at 100 iL/well to wells of the corresponding solid-phase anti-SRSV antibody microplate, followed by a reaction at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction. 10 mM PBS (pH 7.2) which contained "Tween 20" at a final concentration of 0.05% was added at 200 iL/well to the wells, and was then eliminated under suction likewise. This procedure was repeated at least three times. After washing, the POD-labeled anti-SRSV antibody of the corresponding serotype, which had been diluted 20000-fold with a buffer, was added at 100 iL/well, followed by a reaction at room temperature for 60 minutes. Subsequent to washing, a TMB solution with hydrogen peroxide contained therein was added at 100 iL/well, followed by a reaction at room temperature for 30 minutes. After the reaction, 0.6 N sulfuric acid was added at 100 iL/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader. The results are shown in Table 2.

Table 2 Cross-reactivity between Serotypes

Purified VLP	VLP concent-ration (ng/mL)	Solid-phase antibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)										
		124 20000	258 20000	407 20000	645 20000	104 20000	809 20000	754 20000	1876 20000	47 20000	7k 20000	10-25 20000
124	4	1.430	0.018	0.013	0.016	0.013	0.007	0.007	0.008	0.010	0.019	0.009
	0.4	0.192	0.011	0.010	0.011	0.014	0.007	0.007	0.008	0.011	0.018	0.009
	0.04	0.030	0.011	0.011	0.011	0.013	0.007	0.007	0.009	0.012	0.018	0.009
258	4	0.042	1.831	0.114	0.020	0.015	0.009	0.007	0.010	0.012	0.019	0.010
	0.4	0.013	0.270	0.022	0.013	0.016	0.009	0.007	0.009	0.013	0.019	0.011
	0.04	0.008	0.043	0.012	0.011	0.017	0.008	0.007	0.009	0.012	0.018	0.010
407	4	0.084	0.045	0.974	0.010	0.015	0.007	0.007	0.009	0.011	0.018	0.009
	0.4	0.016	0.012	0.134	0.010	0.013	0.007	0.008	0.009	0.011	0.018	0.009
	0.04	0.009	0.010	0.025	0.011	0.014	0.007	0.007	0.008	0.011	0.019	0.009
645	4	0.149	0.034	0.023	0.320	0.016	0.008	0.008	0.009	0.011	0.020	0.010
	0.4	0.024	0.013	0.012	0.045	0.017	0.009	0.008	0.009	0.012	0.019	0.011
	0.04	0.010	0.010	0.011	0.014	0.015	0.009	0.008	0.008	0.012	0.021	0.011
104	4	0.007	0.009	0.009	0.010	0.708	0.007	0.015	0.025	0.017	0.031	0.009
	0.4	0.010	0.009	0.009	0.010	0.094	0.008	0.008	0.011	0.013	0.020	0.009
	0.04	0.009	0.009	0.010	0.011	0.024	0.008	0.007	0.009	0.012	0.020	0.009
809	4	0.013	0.012	0.012	0.011	0.114	0.877	0.047	0.143	0.046	0.080	0.017
	0.4	0.010	0.010	0.011	0.011	0.030	0.134	0.013	0.033	0.018	0.028	0.013
	0.04	0.009	0.010	0.010	0.010	0.017	0.022	0.008	0.011	0.014	0.020	0.011
754	4	0.008	0.011	0.009	0.010	0.038	0.008	0.286	0.068	0.025	0.027	0.013
	0.4	0.008	0.009	0.010	0.011	0.017	0.008	0.038	0.015	0.013	0.020	0.010
	0.04	0.009	0.009	0.011	0.011	0.016	0.008	0.011	0.010	0.012	0.020	0.009
1876	4	0.010	0.012	0.011	0.011	0.026	0.009	0.013	0.728	0.023	0.025	0.012
	0.4	0.009	0.014	0.010	0.011	0.017	0.009	0.008	0.089	0.015	0.021	0.013
	0.04	0.011	0.010	0.010	0.012	0.016	0.010	0.007	0.017	0.014	0.019	0.011
47	4	0.008	0.009	0.009	0.010	0.017	0.007	0.008	0.011	0.324	0.021	0.014
	0.4	0.008	0.009	0.009	0.011	0.015	0.008	0.008	0.009	0.048	0.020	0.013
	0.04	0.008	0.009	0.009	0.011	0.014	0.008	0.008	0.008	0.017	0.022	0.011

Table 2 (Cont'd)

Purified VLP	VLP concent-ration (ng/mL)	Solid-phase amtibody plate x POD (top: strain name, bottom: dilution of POD-labeled antibody)										
		124 20000	258 20000	407 20000	645 20000	104 20000	809 20000	754 20000	1876 20000	47 20000	7k 20000	10-25 20000
7k	4	0.009	0.010	0.010	0.011	0.019	0.009	0.010	0.011	0.015	0.160	0.014
	0.4	0.009	0.011	0.010	0.011	0.016	0.008	0.008	0.008	0.015	0.035	0.016
	0.04	0.011	0.010	0.010	0.011	0.017	0.009	0.008	0.009	0.014	0.022	0.015
10-25	4	0.009	0.010	0.010	0.011	0.098	0.010	0.022	0.069	0.033	0.058	1.050
	0.4	0.007	0.009	0.010	0.011	0.026	0.009	0.009	0.020	0.018	0.026	0.163
	0.04	0.009	0.009	0.009	0.012	0.016	0.009	0.007	0.011	0.015	0.023	0.029
Blank		0.009	0.011	0.010	0.011	0.016	0.009	0.008	0.009	0.017	0.022	0.016

In the table, "645" indicates the Hu/NLV/Kashiwa 645/1999/JP strain, "124" the Hu/NLV/Seto 124/1989/JP strain, "258" the Hu/NLV/Funabashi 258/1996/JP strain, "407" the Hu/NLV/Chiba 407/1987/JP strain, "104" the Hu/NLV/Narita 104/1997/JP strain, "809" the Hu/NLV/Sanbu 809/1998/JP strain, "754" the Hu/NLV/Ichikawa 754/1998/JP strain, "1876" the Hu/NLV/Chitta 1876/1996/JP strain, "47" the Hu/NLV/Kashiwa 47/1997/JP strain, "7k" the Hu/NLV/Mie 7k/1994/JP strain, and "10-25" the Hu/NLV/Osaka 10-25/1999/JP strain.

As a result, no cross-reactivity was observed between viruses of the same genogroup, to say nothing of cross-reactivity between viruses of different Genogroups I and II. It was, therefore, confirmed that the serotypes of the 11 types of used virus strains were different from one another.

Test 1 Discrimination of SRSVs in Genogroup

The anti-SRSV antibodies against the SRSVs belonging to Genogroup I (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, and the Hu/NLV/Chiba 407/1987/JP strain) were diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ìg/mL and were then mixed. The thus-obtained mixture was poured at 100 ìL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4℃. After standing for 18 hours or longer, the microplate was washed 3 to 4 times at 200 ìL/well with PBS which contained "Tween 20" at a final concentration of 0.05%.

10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight at 4℃ to obtain a microplate with the anti-SRSV-IgG antibodies against the respective serotypes of Genogroup I carried in a mixed solid-phase form (Type I plate).

Next, the anti-SRSV antibodies against the SRSVs belonging to Genogroup II (the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were similarly formed into a solid phase to obtain a Type II plate.

To feces (0.5 to 1.0 g) of each SRSV patient, PBS (9 mL) and "Daiflon" (1 mL) were added, followed by homogenization. The thus-prepared suspension was centrifuged under 19,000 g for 20 minutes, and the supernatant was collected and formed into a 10% fecal emulsion. The 10% fecal emulsion was diluted at 1:1 in volume with a buffer. The diluted emulsion was added at 100 iL/well into wells of the Type I and Type II plates, and was allowed to react at room temperature for 60 minutes. After the reaction, the reaction mixtures in the wells were eliminated under suction. 10 mM PBS (pH 7.2) - which contained "Tween 20" at a final concentration of 0.05% - was added at 200 iL/well to the wells, and was then eliminated under suction. This

procedure was performed at least three times. After the washing, the POD-labeled anti-SRSV antibodies of the respective serotypes, said antibodies having had been diluted 20,000-fold with a buffer, were added at 100 iL/well, and were then reacted at room temperature for 60 minutes. After washing, a TMB solution with hydrogen peroxide contained therein was added at 100 iL/well, and were then reacted at room temperature for 30 minutes. Subsequent to the reaction, 0.6 N sulfuric acid was added at 100 iL/well, and the absorbance (450 nm/630 nm) of each well was measured by an ELISA autoreader.

As a result, it was found that among 15 fecal specimens from patients infected to SRSV of Genogroup I, 14 fecal specimens reacted only to the Type I plate and did not react to the Type II plate. Concerning 7 fecal specimens from patients infected to SRSV of Genogroup II, on the other hand, 6 fecal specimens did not react to the Type I plate but reacted only to Type II plate. It has, therefore, been confirmed that discrimination in genogroup is actually feasible.

Test 2 Discrimination of SRSVs in Serotype

The anti-SRSV antibodies against the SRSVs (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the

Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL. The thus-obtained dilutions were poured at 100 iL/well into a polystyrene flat-bottom microplate (manufactured by Nunc). The microplate was allowed to stand overnight at 4℃. After having been allowed to stand for 18 hours or longer, the microplate was washed 3 to 4 times at 200 iL/well with PBS which contained "Tween 20" at a final concentration of 0.05%. 10 mM PBS (pH 7.2) - which contained bovine serum albumin (BSA) and "Tween 20" at final concentrations of 0.5% and 0.05%, respectively - was then added at 200 iL/well. The microplate was allowed to stand overnight at 4℃ to obtain a solid-phase anti-SRSV antibody microplate (serotype discrimination plate).

With respect to fecal specimens from SRSV patients, ELISA was conducted in a similar manner as in Test 1. The results are shown in Table 3.

Table 3 Clinical Test

Total number of specimens: 41

Serotype discriminated by invention kit	Number of detected specimen(s)
HU/NLV/Kashiwa 645/1999/JP	1
Hu/NLV/Seto 124/1989/JP	7
Hu/NLV/Funabashi 258/1996/JP	4
Hu/NLV/Chiba 407/1987/JP	1
HU/NLV/Narita 104/1997/JP	4
Hu/NLV/Sanbu 809/1998/JP	12
Hu/NLV/Ichikawa 754/1998/JP	2
Hu/NLV/Chitta 1876/1996/JP	3
Hu/NLV/Kashiwa 47/1997/JP	1
Hu/NLV/Mie 7k/1994/JP	1
Hu/NLV/Osaka 10-25/1999/JP	2
Total number of detected specimens	38 (93%)

As a result, it has been found that according to the SRSV detection method of the present invention, SRSVs can be detected with a probability as high as 93% and their serotypes can also be discriminated.

Further, the serotypes discriminated by the kit of the present invention were consistent with those ascertained by PCR and an analysis of their base sequences (Table 4).

Table 4 Ascertainment of Serotypes

Total number of specimens: 38

Serotype discriminated by invention kit		Number of specimen(s) discriminated in serotype by PCR and analysis of base sequences
HU/NLV/Kashiwa 645/1999/JP	1 Specimens	1
Hu/NLV/Seto 124/1989/JP	7 Specimens	7
Hu/NLV/Funabashi 258/1996/JP	4 Specimens	4
Hu/NLV/Chiba 407/1987/JP	1 Specimen	1
HU/NLV/Narita 104/1997/JP	4 Specimens	4
Hu/NLV/Sanbu 809/1998/JP	12 Specimens	12
Hu/NLV/Ichikawa 754/1998/JP	2 Specimens	2
Hu/NLV/Chitta 1876/1996/JP	3 Specimens	3
Hu/NLV/Kashiwa 47/1997/JP	1 Specimen	1
Hu/NLV/Mie 7k/1994/JP	1 Specimen	1
Hu/NLV/Osaka 10-25/1999/JP	2 Specimens	2

The anti-SRSV antibodies against the SRSVs (the Hu/NLV/Kashiwa 645/1999/JP strain, the Hu/NLV/Seto 124/1989/JP strain, the Hu/NLV/Funabashi 258/1996/JP strain, the Hu/NLV/Chiba 407/1987/JP strain, the Hu/NLV/Narita 104/1997/JP strain, the Hu/NLV/Sanbu 809/1998/JP strain, the Hu/NLV/Ichikawa 754/1998/JP strain, the Hu/NLV/Chitta 1876/1996/JP strain, the Hu/NLV/Kashiwa 47/1997/JP strain, the Hu/NLV/Mie 7k/1994/JP strain, and the Hu/NLV/Osaka 10-25/1999/JP strain) were each independently diluted with a

carbonate buffer (pH 9.5) to a concentration of from 0.5 to 10 ig/mL. All the dilutions so obtained were mixed. As an alternative, the anti-SRSV antibodies may be diluted after mixing them together. Using the thus-diluted mixture of the anti-SRSV antibodies, a solid-phase anti-SRSV antibody microplate was produced likewise. With respect to 22 fecal specimens from patients infected to SRSV, ELISA was conducted in a similar manner as in Test 1. It was possible to detect SRSV in 20 specimens.

Industrial Applicability

According to the SRSV detection kit of the present invention, it is possible to detect most of the SRSV-related viruses discovered to date and also to discriminate their serotypes and genogroups. When SRSV-related food poisoning occurs, the SRSV detection kit of the present invention is, therefore, useful for specifying an infection route, preventing the infection from spreading, and performing an epidemiological investigation.

CLAIMS

1. An SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented

by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

2. An SRSV detection kit according to claim 1, wherein said antibodies have been prepared by immunizing with virus-like particles.

3. An SRSV detection kit according to claim 1, which is useful for distinguishing serotype of SRSVs.

4. An SRSV detection kit for discriminating genogroup of SRSVs, the kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (d), respectively:

(a) a peptide having an amino acid sequence represented by SEQ ID NO: 1 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(b) a peptide having an amino acid sequence represented by SEQ ID NO: 2 and peptides each having at least 80% of homology

with said amino acid sequence, and partial peptides thereof,

(c) a peptide having an amino acid sequence represented by SEQ ID NO: 3 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(d) a peptide having an amino acid sequence represented by SEQ ID NO: 4 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

5. An SRSV detection kit for discriminating genogroup of SRSVs, the kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (e) to (k), respectively:

(e) a peptide having an amino acid sequence represented by SEQ ID NO: 5 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(f) a peptide having an amino acid sequence represented by SEQ ID NO: 6 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(g) a peptide having an amino acid sequence represented by SEQ ID NO: 7 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(h) a peptide having an amino acid sequence represented by SEQ ID NO: 8 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof,

(i) a peptide having an amino acid sequence represented by SEQ ID NO: 9 and peptides each having at least 80% of homology

with said amino acid sequence, and partial peptides thereof,

(j) a peptide having an amino acid sequence represented by SEQ ID NO: 10 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof, and

(k) a peptide having an amino acid sequence represented by SEQ ID NO: 11 and peptides each having at least 80% of homology with said amino acid sequence, and partial peptides thereof.

6. An SRSV detection kit according to claims 1-5, wherein SRSVs are captured with solid-phase antibody carriers having said antibodies immobilized thereon.

7. An Hu/NLV/Chiba/407/1987/JP gene having a base sequence represented by SEQ ID NO: 15 or a base sequence similar to the first-mentioned base sequence except for deletion, replacement or addition of one to several bases of said first-mentioned base sequence.

8. An Hu/NLV/Kashiwa 47/1997/JP gene having a base sequence represented by SEQ ID NO: 20 or a base sequence similar to the first-mentioned base sequence except for deletion, replacement or addition of one to several bases of said first-mentioned base sequence.

9. An Hu/NLV/Mie 7k/1994/JP gene having a base sequence represented by SEQ ID NO: 21 or a base sequence similar to the first-mentioned base sequence except for deletion, replacement or addition of one to several bases of said first-mentioned base sequence.

10. An Hu/NLV/Osaka 10-25/1999/JP gene having a base sequence represented by SEQ ID NO: 22 or a base sequence similar to the first-mentioned base sequence except for deletion, replacement or addition of one to several bases of said first-mentioned base sequence.

ABSTRACT

This invention relates to an SRSV detection kit comprising all antibodies against SRSV-related virus constituting peptides selected from the following peptide groups (a) to (k), respectively: (a) a peptide having an amino acid sequence represented by SEQ ID NO: 1, and the like, (b) a peptide having an amino acid sequence represented by SEQ ID NO: 2, and the like, (c) a peptide having an amino acid sequence represented by SEQ ID NO: 3, and the like, (d) a peptide having an amino acid sequence represented by SEQ ID NO: 4, and the like, (e) a peptide having an amino acid sequence represented by SEQ ID NO: 5, and the like, (f) a peptide having an amino acid sequence represented by SEQ ID NO: 6, and the like, (g) a peptide having an amino acid sequence represented by SEQ ID NO: 7, and the like, (h) a peptide having an amino acid sequence represented by SEQ ID NO: 8, and the like, (i) a peptide having an amino acid sequence represented by SEQ ID NO: 9, and the like, (j) a peptide having an amino acid sequence represented by SEQ ID NO: 10, and the like, and (k) a peptide having an amino acid sequence represented by SEQ ID NO: 11, and the like.

Use of the kit makes it possible to detect most SRSV-related viruses and further to distinguish their serotypes and genogroups, easily and surely.

FIG. 1

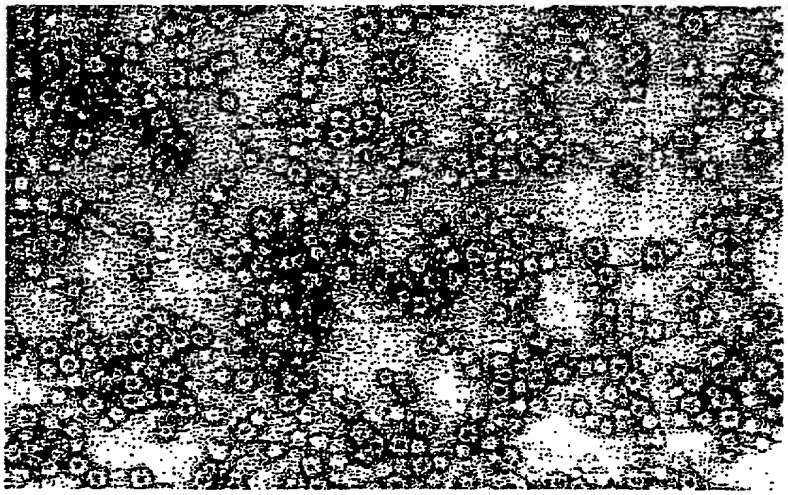


FIG. 2

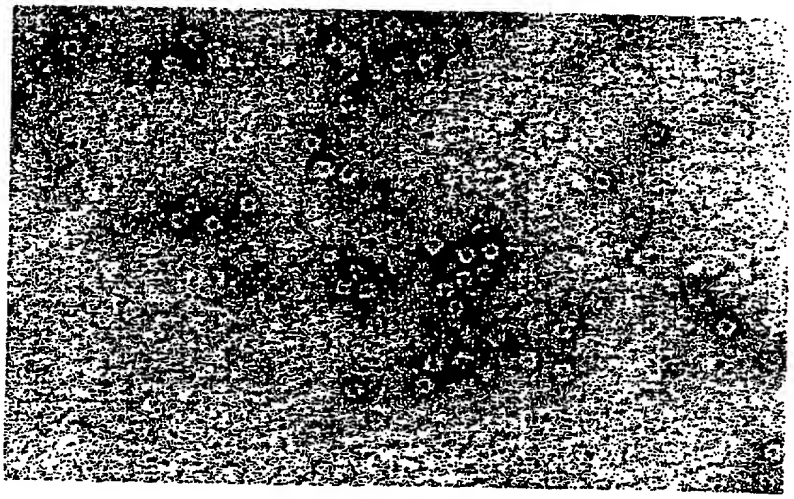


FIG. 3

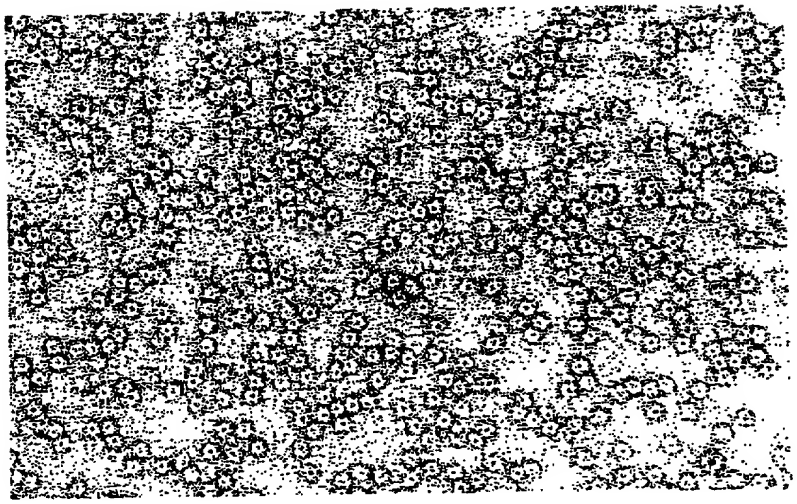
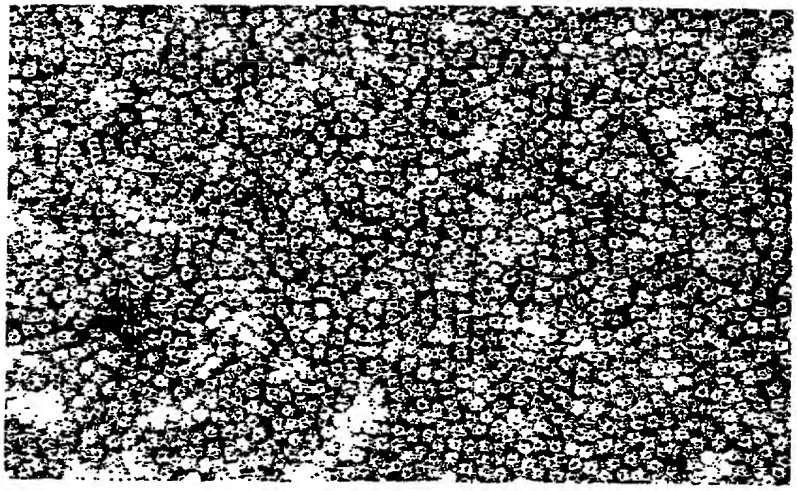


FIG. 4



09/926799

FIG. 5

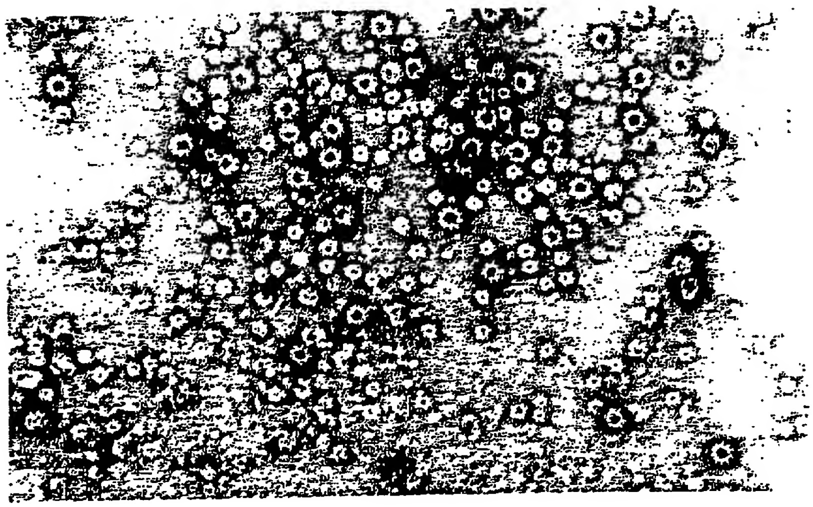


FIG. 6

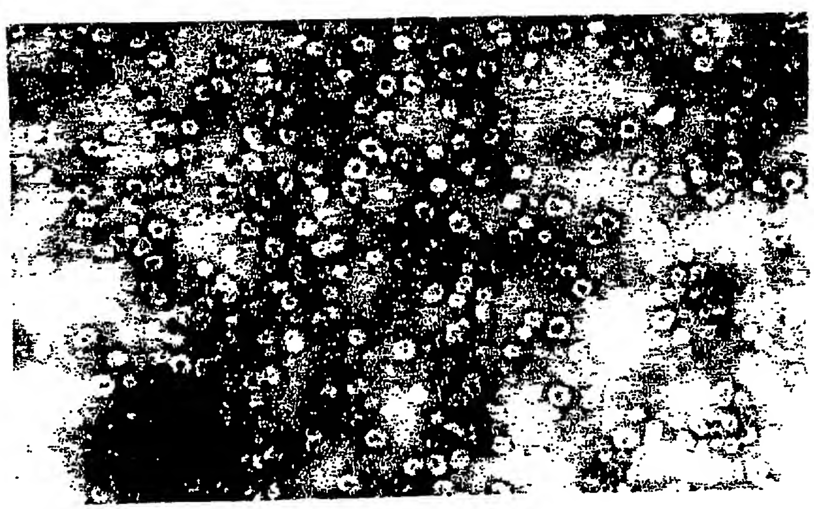


FIG. 7

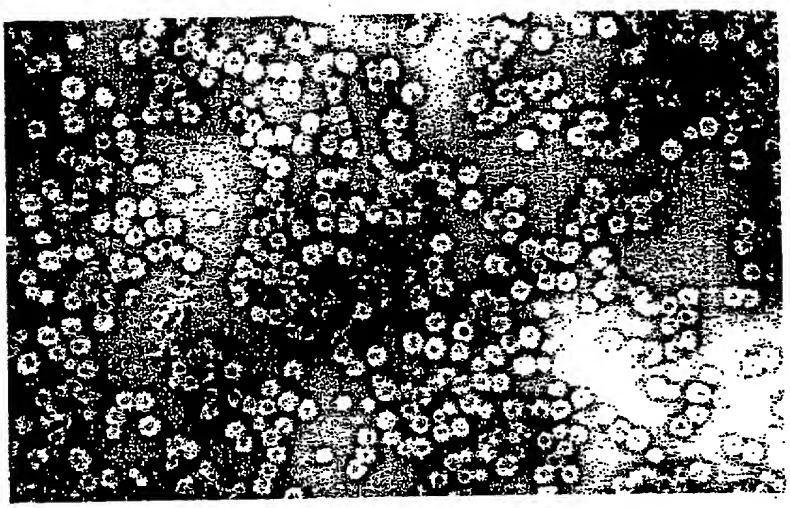


FIG. 8

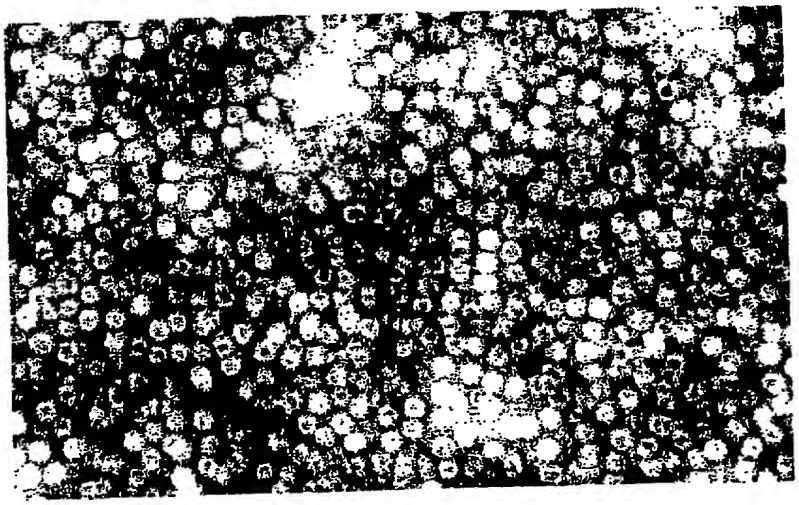


FIG. 9

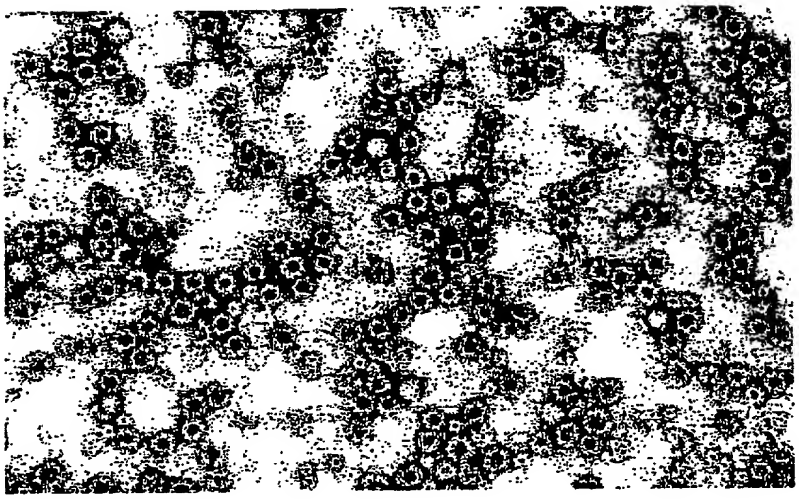
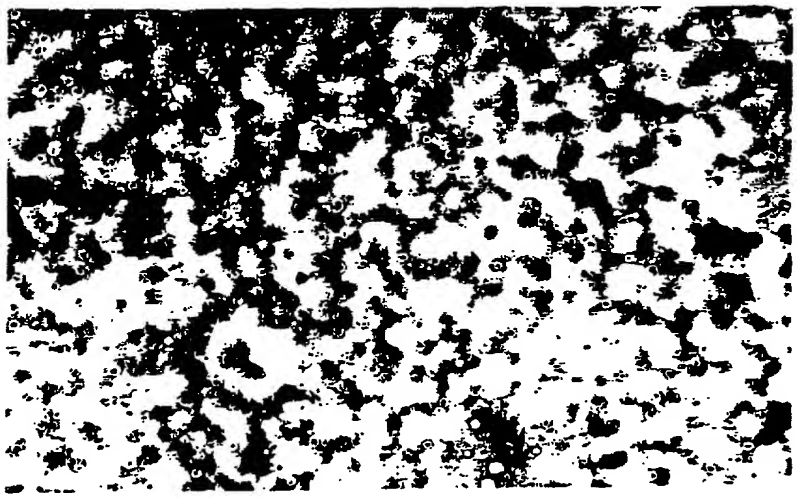


FIG. 10

09/926799

FIG. 11



Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

DK0001 USA ①

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

ＳＲＳＶ検出キット

SRSV DETECTION KIT

上記発明の明細書は、

the specification of which

☐ 本書に添付されています。

☐ is attached hereto.

☒ 2000年6月22日に提出され、米国出願番号または特許協定条約国際出願番号を PCT/JP00/04095 とし、

☒ was filed on June 22, 2000 as ~~United States Application Number~~ or PCT International Application Number PCT/JP00/04095 and was amended on _____ (if applicable).

（該当する場合）_____に訂正されました。

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第35編119条（a）-（d）項又は365条（b）項に基づき下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約365（a）項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Claimed 優先権主張
11-175928	Japan	22/06/1999	☒ Yes はい ☐ No いいえ
			☐ Yes はい ☐ No いいえ

私は、第35編米国法典119条（e）項に基づいて下記の米国特許出願規定に記載された権利をここに主張いたします。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第35編120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約365条（c）に基づく権利をここに主張します。また、本出願の各請求範囲の内容が米国法典第35編112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内または特許協力条約国際提出日までの期間中に入手された、連邦規則法典第37編1条56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration
(日本語宣言書)

委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。
(弁護士、または代理人の指名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

022850

書類送付先

Send Correspondence to:

022850

直接電話連絡先：（名前及び電話番号）

Direct Telephone Calls to: (name and telephone number)

(703) 413-3000

単独発明者または第一の共同発明者の氏名 武田 直和 1-00	Full name of sole or first joint inventor Naokazu TAKEDA
発明者の署名 Naokazu Takeda 日付 Nov. 9, 2001	Inventor's signature Date
住所 162-0052 日本国東京都新宿区戸山一丁目23番1号 国立感染症研究所内	Residence C/O NATIONAL INSTITUTE OF INFECTIOUS DISEASES 23-1, TOYAMA 1-CHOME, SHINJUKU-KU, TOKYO 162-0052 JAPAN JPK
国籍 日本国	Citizenship JAPANESE
郵便の宛先 住所に同じ	Post Office Address SAME AS ABOVE
第二の共同発明者の氏名 2-00 名取 克郎	Full name of second joint inventor, if any Katsuro NATORI
第二の共同発明者の署名 Katsuro Natori 日付 Nov. 9, 2001	Second joint Inventor's signature Date
住所 162-0052 日本国東京都新宿区戸山一丁目23番1号 国立感染症研究所内	Residence C/O NATIONAL INSTITUTE OF INFECTIOUS DISEASES 23-1, TOYAMA 1-CHOME, SHINJUKU-KU, TOKYO 162-0052 JAPAN
国籍 日本国	Citizenship JPK
郵便の宛先 住所に同じ	Post Office Address JAPANESE SAME AS ABOVE

(第三以降の共同発明者についても同様に記載し、署名すること)

(Supply similar information and signature for third and subsequent joint inventors.)

1/01

Japanese Language Declaration
(日本語宣言書)

第三の共同発明者の氏名 宮村 達男 3rd	Full name of third joint inventor, if any Tatsuo MIYAMURA
第三の共同発明者の署名 Tatsuo Miyamura 日付 Nov. 9, 2001	Third joint Inventor's signature Date
住所 162-0052 日本国東京都新宿区戸山一丁目23番1号 国立感染症研究所内	Residence C/O NATIONAL INSTITUTE OF INFECTIOUS DISEASES 23-1, TOYAMA 1-CHOME, SHINJUKU-KU, TOKYO 162-0052 JAPAN
国籍 日本国	Citizenship JAPANESE JPX
郵便の宛先 住所に同じ	Post Office Address SAME AS ABOVE

第四の共同発明者の氏名 鎌田 公仁夫 4th	Full name of fourth joint inventor, if any Kunio KAMATA
第四の共同発明者の署名 Kunio Kamata 日付 Oct. 29, 2001	Fourth joint Inventor's signature Date
住所 959-1836 日本国新潟県五泉市南本町一丁目2番2号 デンカ生研株式会社内	Residence C/O DENKA SEIKEN CO., LTD., 2-2, MINAMIHONCHO 1-CHOME, GOSEN-SHI, NIIGATA 959-1836 JAPAN
国籍 日本国	Citizenship JAPANESE JPX
郵便の宛先 住所に同じ	Post Office Address SAME AS ABOVE

第五の共同発明者の氏名 佐藤 俊則 5th	Full name of fifth joint inventor, if any Toshinori SATO
第五の共同発明者の署名 Toshinori Sato 日付 Oct. 31, 2001	Fifth joint Inventor's signature Date
住所 959-1836 日本国新潟県五泉市南本町一丁目2番2号 デンカ生研株式会社内	Residence C/O DENKA SEIKEN CO., LTD., 2-2, MINAMIHONCHO 1-CHOME, GOSEN-SHI, NIIGATA 959-1836 JAPAN
国籍 日本国	Citizenship JAPANESE JPX
郵便の宛先 住所に同じ	Post Office Address SAME AS ABOVE

第六の共同発明者の氏名 佐藤 征也 6th	Full name of sixth joint inventor, if any Seiya SATO
第六の共同発明者の署名 Seiya Sato 日付 Oct 31 2001	Sixth joint Inventor's signature Date
住所 959-1836 日本国新潟県五泉市南本町一丁目2番2号 デンカ生研株式会社内	Residence C/O DENKA SEIKEN CO., LTD., 2-2, MINAMIHONCHO 1-CHOME, GOSEN-SHI, NIIGATA 959-1836 JAPAN
国籍 日本国	Citizenship JAPANESE JPX
郵便の宛先 住所に同じ	Post Office Address SAME AS ABOVE

(第六またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。)

(Supply similar information and signature for third and subsequent joint inventors.)

SEQUENCE LISTING

<110> DENKA SEIKEN CO., LTD
National Institute of Infectious Diseases

<120> Detection Kit for SRSV

<130> DK0001

<150> JP P1999-175928
<151> 1999-06-22

<160> 34

<170> PatentIn Ver. 2.1

<210> 1
<211> 545
<212> PRT
<213> Hu/NLV/Kashiwa 645/1999/JP

<400> 1

```
Met Met Met Ala Ser Lys Asp Ala Pro Thr Asn Met Asp Gly Thr Ser
  1               5                  10                  15

Gly Ala Gly Gln Leu Val Pro Glu Ala Asn Thr Ala Glu Pro Ile Ser
             20                  25                  30

Met Glu Pro Val Ala Gly Ala Ala Thr Ala Ala Ala Thr Ala Gly Gln
         35                  40                  45

Val Asn Met Ile Asp Pro Trp Ile Met Asn Asn Tyr Val Gln Ala Pro
     50                  55                  60

Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Ile Leu
 65                  70                  75                  80

Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
                 85                  90                  95

Ala Gln Met Tyr Asn Gly Trp Val Gly Asn Met Lys Val Lys Val Leu
            100                 105                 110

Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Ile Ile Ile Ser Cys Ile
        115                 120                 125
```

Pro Pro Gly Phe Ala Ala Gln Asn Ile Ser Ile Ala Gln Ala Thr Met
130 135 140

Phe Pro His Val Ile Ala Asp Val Arg Val Leu Glu Pro Ile Glu Val
145 150 155 160

Pro Leu Glu Asp Val Arg Asn Val Leu Phe His Asn Asn Asp Asn Ala
165 170 175

Pro Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu Arg Ala Ser
180 185 190

Gly Ser Ser Ser Gly Thr Asp Pro Phe Val Ile Ala Gly Arg Val Leu
195 200 205

Thr Cys Pro Ser Pro Asp Phe Ser Phe Leu Phe Leu Val Pro Pro Asn
210 215 220

Val Glu Gln Lys Thr Lys Pro Phe Ser Val Pro Asn Leu Pro Leu Asn
225 230 235 240

Thr Leu Ser Asn Ser Arg Val Pro Ser Leu Ile Lys Ser Met Met Val
245 250 255

Ser Arg Asp His Gly Gln Met Val Gln Phe Gln Asn Gly Arg Val Thr
260 265 270

Leu Asp Gly Gln Leu Gln Gly Thr Thr Pro Thr Ser Ala Ser Gln Leu
275 280 285

Cys Lys Ile Arg Gly Ser Val Phe His Ala Asn Gly Gly Asn Gly Tyr
290 295 300

Asn Leu Thr Glu Leu Asp Gly Ser Pro Tyr His Ala Phe Glu Ser Pro
305 310 315 320

Ala Pro Ile Gly Phe Pro Asp Leu Gly Glu Cys Asp Trp His Met Glu
325 330 335

Ala Ser Pro Thr Thr Gln Phe Asn Thr Gly Asp Val Ile Lys Gln Ile
340 345 350

Asn Val Lys Gln Glu Ser Ala Phe Ala Pro His Leu Gly Thr Ile Gln
355 360 365

Ala Asp Gly Leu Ser Asp Val Ser Val Asn Thr Asn Met Ile Ala Lys

370 375 380

Leu Gly Trp Val Ser Pro Val Ser Asp Gly His Arg Gly Asp Val Asp
385 390 395 400

Pro Trp Val Ile Pro Arg Tyr Gly Ser Thr Leu Thr Glu Ala Ala Gln
405 410 415

Leu Ala Pro Pro Ile Tyr Pro Pro Gly Phe Gly Glu Ala Ile Val Phe
420 425 430

Phe Met Ser Asp Phe Pro Ile Ala His Gly Thr Asn Gly Leu Ser Val
435 440 445

Pro Cys Thr Ile Pro Gln Glu Phe Val Thr His Phe Val Asn Glu Gln
450 455 460

Ala Pro Thr Arg Gly Glu Ala Ala Leu Leu His Tyr Leu Asp Pro Asp
465 470 475 480

Thr His Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Glu Gly Phe Met
485 490 495

Thr Cys Val Pro Asn Ser Ser Gly Thr Gly Pro Gln Thr Leu Pro Ile
500 505 510

Asn Gly Val Phe Val Phe Val Ser Trp Val Ser Arg Phe Tyr Gln Leu
515 520 525

Lys Pro Val Gly Thr Ala Gly Pro Ala Cys Arg Leu Gly Ile Arg Arg
530 535 540

Ser
545

<210> 2
<211> 530
<212> PRT
<213> Hu/NLV/Seto 124/1989/JP

<400> 2
Met Met Met Ala Ser Lys Asp Ala Thr Ser Ser Val Asp Gly Ala Ser
1 5 10 15

Gly Ala Gly Gln Leu Val Pro Glu Val Asn Ala Ser Asp Pro Leu Ala

```
                20                  25                  30

Met Asp Pro Val Ala Gly Ser Ser Thr Ala Val Ala Thr Ala Gly Gln
            35                  40                  45

Val Asn Pro Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln Ala Pro
        50                  55                  60

Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Gly Val Leu
65                  70                  75                  80

Phe Asp Leu Ser Leu Gly Pro His Leu Asn Pro Phe Leu Leu His Leu
                85                  90                  95

Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg Ile Met
            100                 105                 110

Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Ile Ile Val Ser Cys Ile
        115                 120                 125

Pro Pro Gly Phe Gly Ser His Asn Leu Thr Ile Ala Gln Ala Thr Leu
    130                 135                 140

Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Asp Pro Ile Glu Val
145                 150                 155                 160

Pro Leu Glu Asp Val Arg Asn Val Leu Phe His Asn Asn Asp Arg Asn
                165                 170                 175

Gln Gln Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu Arg Thr
            180                 185                 190

Gly Gly Gly Thr Gly Asp Ser Phe Val Val Ala Gly Arg Val Met Thr
        195                 200                 205

Cys Pro Ser Pro Asp Phe Asn Phe Leu Phe Leu Val Pro Pro Thr Val
    210                 215                 220

Glu Gln Lys Thr Arg Pro Phe Thr Leu Pro Asn Leu Pro Leu Ser Ser
225                 230                 235                 240

Leu Ser Asn Ser Arg Ala Pro Leu Pro Ile Ser Gly Met Gly Ile Ser
                245                 250                 255

Pro Asp Asn Val Gln Ser Val Gln Phe Gln Asn Gly Arg Cys Thr Leu
            260                 265                 270
```

Asp Gly Arg Leu Val Gly Thr Thr Pro Val Ser Leu Ser His Val Ala
275 280 285

Lys Ile Arg Gly Thr Ser Asn Gly Thr Val Ile Asn Leu Thr Glu Leu
290 295 300

Asp Gly Thr Pro Phe His Pro Phe Glu Gly Pro Ala Pro Ile Gly Phe
305 310 315 320

Pro Asp Leu Gly Gly Cys Asp Trp His Ile Asn Met Thr Gln Phe Gly
325 330 335

His Ser Ser Gln Thr Gln Tyr Asp Val Asp Thr Thr Pro Asp Thr Phe
340 345 350

Val Pro His Leu Gly Ser Ile Gln Ala Asn Gly Ile Gly Ser Gly Asn
355 360 365

Tyr Ile Gly Val Leu Ser Trp Val Ser Pro Pro Ser His Pro Ser Gly
370 375 380

Ser Gln Val Asp Leu Trp Lys Ile Pro Asn Tyr Gly Ser Ser Ile Thr
385 390 395 400

Glu Ala Thr His Leu Ala Pro Ser Val Tyr Pro Pro Gly Phe Gly Glu
405 410 415

Val Leu Val Phe Phe Met Ser Lys Ile Pro Gly Pro Gly Ala Tyr Ser
420 425 430

Leu Pro Cys Leu Leu Pro Gln Glu Tyr Ile Ser His Leu Ala Ser Glu
435 440 445

Gln Ala Pro Thr Val Gly Glu Ala Ala Leu Leu His Tyr Val Asp Pro
450 455 460

Asp Thr Gly Arg Thr Leu Gly Glu Phe Lys Ala Tyr Pro Asp Gly Phe
465 470 475 480

Leu Thr Cys Val Pro Asn Gly Ala Ser Ser Gly Pro Gln Gln Leu Pro
485 490 495

Ile Asn Gly Val Phe Val Phe Val Ser Trp Val Ser Arg Phe Tyr Gln
500 505 510

Leu Lys Pro Val Gly Thr Ala Ser Ser Ala Arg Gly Arg Leu Gly Leu
515 520 525

Arg Arg
530

<210> 3
<211> 545
<212> PRT
<213> Hu/NLV/Funabashi 258/1996/JP

<400> 3
Met Met Met Ala Ser Lys Asp Ala Pro Gln Ser Ala Asp Gly Ala Ser
1 5 10 15

Gly Ala Gly Gln Leu Val Pro Glu Val Asn Thr Ala Asp Pro Leu Pro
20 25 30

Met Glu Pro Val Ala Gly Pro Thr Thr Ala Val Ala Thr Ala Gly Gln
35 40 45

Val Asn Met Ile Asp Pro Trp Ile Val Asn Asn Phe Val Gln Ser Pro
50 55 60

Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Ile Leu
65 70 75 80

Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
85 90 95

Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg Ile Leu
100 105 110

Leu Ala Gly Asn Ala Phe Ser Ala Gly Lys Ile Ile Val Cys Cys Val
115 120 125

Pro Pro Gly Phe Thr Ser Ser Ser Leu Thr Ile Ala Gln Ala Thr Leu
130 135 140

Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Glu Pro Ile Glu Met
145 150 155 160

Pro Leu Glu Asp Val Arg Asn Val Leu Tyr His Thr Asn Asp Asn Gln
165 170 175

Pro Thr Met Arg Leu Val Cys Met Leu Tyr Thr Pro Leu Arg Thr Gly
180 185 190

Gly Gly Ser Gly Asn Ser Asp Ser Phe Val Val Ala Gly Arg Val Leu
195 200 205

Thr Ala Pro Ser Ser Asp Phe Ser Phe Leu Phe Leu Val Pro Pro Thr
210 215 220

Ile Glu Gln Lys Thr Arg Ala Phe Thr Val Pro Asn Ile Pro Leu Gln
225 230 235 240

Thr Leu Ser Asn Ser Arg Phe Pro Ser Leu Ile Gln Gly Met Ile Leu
245 250 255

Ser Pro Asp Ala Ser Gln Val Val Gln Phe Gln Asn Gly Arg Cys Leu
260 265 270

Ile Asp Gly Gln Leu Leu Gly Thr Thr Pro Ala Thr Ser Gly Gln Leu
275 280 285

Phe Arg Val Arg Gly Lys Ile Asn Gln Gly Ala Arg Thr Leu Asn Leu
290 295 300

Thr Glu Val Asp Gly Lys Pro Phe Met Ala Phe Asp Ser Pro Ala Pro
305 310 315 320

Val Gly Phe Pro Asp Phe Gly Lys Cys Asp Trp His Met Arg Ile Ser
325 330 335

Lys Thr Pro Asn Asn Thr Ser Ser Gly Asp Pro Met Arg Ser Val Ser
340 345 350

Val Gln Thr Asn Val Gln Gly Phe Val Pro His Leu Gly Ser Ile Gln
355 360 365

Phe Asp Glu Val Phe Asn His Pro Thr Gly Asp Tyr Ile Gly Thr Ile
370 375 380

Glu Trp Ile Ser Gln Pro Ser Thr Pro Pro Gly Thr Asp Ile Asn Leu
385 390 395 400

Trp Glu Ile Pro Asp Tyr Gly Ser Ser Leu Ser Gln Ala Ala Asn Leu
405 410 415

Ala Pro Pro Val Phe Pro Pro Gly Phe Gly Glu Ala Leu Val Tyr Phe

420 425 430

Val Ser Ala Phe Pro Gly Pro Asn Asn Arg Ser Ala Pro Asn Asp Val
435 440 445

Pro Cys Leu Leu Pro Gln Glu Tyr Ile Thr His Phe Val Ser Glu Gln
450 455 460

Ala Pro Thr Met Gly Asp Ala Ala Leu Leu His Tyr Val Asp Pro Asp
465 470 475 480

Thr Asn Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Gly Gly Tyr Leu
485 490 495

Thr Cys Val Pro Asn Gly Val Gly Ala Gly Pro Gln Gln Leu Pro Leu
500 505 510

Asn Gly Val Phe Leu Phe Val Ser Trp Val Ser Arg Phe Tyr Gln Leu
515 520 525

Lys Pro Val Gly Thr Ala Ser Thr Ala Arg Ser Arg Leu Gly Val Arg
530 535 540

Arg Ile
545

<210> 4
<211> 544
<212> PRT
<213> Hu/NLV/Chiba 407/1987/JP

<400> 4
Met Met Met Ala Ser Lys Asp Ala Thr Pro Ser Ala Asp Gly Ala Thr
1 5 10 15

Gly Ala Gly Gln Leu Val Pro Glu Val Asn Thr Ala Asp Pro Ile Pro
20 25 30

Ile Asp Pro Val Ala Gly Ser Ser Thr Ala Leu Ala Thr Ala Gly Gln
35 40 45

Val Asn Leu Ile Asp Pro Trp Ile Ile Asn Asn Phe Val Gln Ala Pro
50 55 60

Gln Gly Glu Phe Thr Ile Ser Pro Asn Asn Thr Pro Gly Asp Val Leu

65 70 75 80

Phe Asp Leu Gln Leu Gly Pro His Leu Asn Pro Phe Leu Ser His Leu
85 90 95

Ser Gln Met Tyr Asn Gly Trp Val Gly Asn Met Arg Val Arg Val Val
100 105 110

Leu Ala Gly Asn Ala Phe Thr Ala Gly Lys Val Ile Ile Cys Cys Val
115 120 125

Pro Pro Gly Phe Gln Ser Arg Thr Leu Ser Ile Ala Gln Ala Thr Leu
130 135 140

Phe Pro His Val Ile Ala Asp Val Arg Thr Leu Asp Pro Val Glu Val
145 150 155 160

Pro Leu Glu Asp Val Arg Asn Val Leu Tyr His Asn Asn Asp Thr Gln
165 170 175

Pro Thr Met Arg Leu Leu Cys Met Leu Tyr Thr Pro Leu Arg Thr Gly
180 185 190

Gly Ala Ser Gly Gly Thr Asp Ser Phe Val Val Ala Gly Arg Val Leu
195 200 205

Thr Cys Pro Gly Pro Asp Phe Asn Phe Leu Phe Leu Val Pro Pro Thr
210 215 220

Val Glu Gln Lys Thr Arg Pro Phe Thr Val Pro Asn Ile Pro Leu Lys
225 230 235 240

Tyr Leu Ser Asn Ser Arg Ile Pro Asn Pro Ile Glu Gly Met Ser Leu
245 250 255

Ser Pro Asp Gln Thr Gln Asn Val Gln Phe Gln Asn Gly Arg Cys Thr
260 265 270

Ile Asp Gly Gln Pro Leu Gly Thr Thr Pro Val Ser Val Ser Gln Leu
275 280 285

Cys Lys Phe Arg Gly Arg Ile Thr Ser Gly Gln Arg Val Leu Asn Leu
290 295 300

Thr Glu Leu Asp Gly Ser Pro Phe Met Ala Phe Ala Ala Pro Ala Pro
305 310 315 320

Ala Gly Phe Pro Asp Leu Gly Ser Cys Asp Trp His Ile Glu Met Ser
325 330 335

Lys Ile Pro Asn Ser Ser Thr Gln Asn Asn Pro Ile Val Thr Asn Ser
340 345 350

Val Lys Pro Asn Ser Gln Gln Phe Val Pro His Leu Ser Ser Ile Thr
355 360 365

Leu Asp Glu Asn Val Ser Ser Gly Gly Asp Tyr Ile Gly Thr Ile Gln
370 375 380

Trp Thr Ser Pro Pro Ser Asp Ser Gly Gly Ala Asn Thr Asn Phe Trp
385 390 395 400

Lys Ile Pro Asp Tyr Gly Ser Ser Leu Ala Glu Ala Ser Gln Leu Ala
405 410 415

Pro Ala Val Tyr Pro Pro Gly Phe Asn Glu Val Ile Val Tyr Phe Met
420 425 430

Ala Ser Ile Pro Gly Pro Asn Gln Ser Gly Ser Pro Asn Leu Val Pro
435 440 445

Cys Leu Leu Pro Gln Glu Tyr Ile Thr His Phe Ile Ser Glu Gln Ala
450 455 460

Pro Ile Gln Gly Glu Ala Ala Leu Leu His Tyr Val Asp Pro Asp Thr
465 470 475 480

Asn Arg Asn Leu Gly Glu Phe Lys Leu Tyr Pro Gly Gly Tyr Leu Thr
485 490 495

Cys Val Pro Asn Ser Ser Ser Thr Gly Pro Gln Gln Leu Pro Leu Asp
500 505 510

Gly Val Phe Val Phe Ala Ser Trp Val Ser Arg Phe Tyr Gln Leu Lys
515 520 525

Pro Val Gly Thr Ala Gly Pro Ala Arg Gly Arg Leu Gly Val Arg Arg
530 535 540

<210> 5
<211> 539
<212> PRT
<213> Hu/NLV/Narita 104/1997/JP

<400> 5

```
Met Lys Met Ala Ser Asn Asp Ala Asn Pro Ser Asp Gly Ser Thr Ala
  1               5                  10                  15

Asn Leu Val Pro Glu Val Asn Asn Glu Val Met Ala Leu Glu Pro Val
             20                  25                  30

Val Gly Ala Ala Ile Ala Ala Pro Val Ala Gly Gln Gln Asn Val Ile
         35                  40                  45

Asp Pro Trp Ile Arg Asn Asn Phe Val Gln Ala Pro Gly Gly Glu Phe
     50                  55                  60

Thr Val Ser Pro Arg Asn Ala Pro Gly Glu Ile Leu Trp Ser Ala Pro
 65                  70                  75                  80

Leu Gly Pro Asp Leu Asn Pro Tyr Leu Ser His Leu Ala Arg Met Tyr
                 85                  90                  95

Asn Gly Tyr Ala Gly Gly Phe Glu Val Gln Val Ile Leu Ala Gly Asn
            100                 105                 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Val Pro Pro Asn Phe
        115                 120                 125

Pro Thr Glu Gly Leu Ser Pro Ser Gln Val Thr Met Phe Pro His Ile
    130                 135                 140

Ile Val Asp Val Arg Gln Leu Glu Pro Val Leu Ile Pro Leu Pro Asp
145                 150                 155                 160

Val Arg Asn Asn Phe Tyr His Tyr Asn Gln Ser Asn Asp Ser Thr Ile
                165                 170                 175

Lys Leu Ile Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Asn Ala Gly
            180                 185                 190

Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ser Pro
        195                 200                 205
```

Asp Phe Asp Phe Ile Phe Leu Val Pro Pro Thr Val Glu Ser Arg Thr
210 215 220

Lys Pro Phe Thr Val Pro Ile Leu Thr Val Glu Glu Met Ser Asn Ser
225 230 235 240

Arg Phe Pro Ile Pro Leu Glu Lys Leu Tyr Thr Gly Pro Ser Ser Ala
245 250 255

Phe Val Val Gln Pro Gln Asn Gly Arg Cys Thr Thr Asp Gly Val Leu
260 265 270

Leu Gly Thr Thr Gln Leu Ser Ala Val Asn Ile Cys Thr Phe Arg Gly
275 280 285

Asp Val Thr His Ile Ala Gly Ser His Asp Tyr Thr Met Asn Leu Ala
290 295 300

Ser Gln Asn Trp Ser Asn Tyr Asp Pro Thr Glu Glu Ile Pro Ala Pro
305 310 315 320

Leu Gly Thr Pro Asp Phe Val Gly Lys Ile Gln Gly Met Leu Thr Gln
325 330 335

Thr Thr Arg Glu Asp Gly Ser Thr Arg Ala His Lys Ala Thr Val Ser
340 345 350

Thr Gly Ser Val His Phe Thr Pro Lys Leu Gly Ser Val Gln Tyr Thr
355 360 365

Thr Asp Thr Asn Asn Asp Phe Gln Thr Gly Gln Asn Thr Lys Phe Thr
370 375 380

Pro Val Gly Val Ile Gln Asp Gly Asn Asn His Gln Asn Glu Pro Gln
385 390 395 400

Gln Trp Val Leu Pro Asn Tyr Ser Gly Arg Thr Gly His Asn Val His
405 410 415

Leu Ala Pro Ala Val Ala Pro Thr Phe Pro Gly Glu Gln Leu Leu Phe
420 425 430

Phe Arg Ser Thr Met Pro Gly Cys Ser Gly Tyr Pro Asn Met Asn Leu
435 440 445

Asp Cys Leu Leu Pro Gln Glu Trp Val Gln His Phe Cys Gln Glu Ala

```
    450                 455                 460

Ala Pro Ala Gln Ser Asp Val Ala Leu Leu Arg Phe Val Asn Pro Asp
465                 470                 475                 480

Thr Gly Arg Val Leu Phe Glu Cys Lys Leu His Lys Ser Gly Tyr Val
                485                 490                 495

Thr Val Ala His Thr Gly Pro His Asp Leu Val Ile Pro Pro Asn Gly
            500                 505                 510

Tyr Phe Arg Phe Asp Ser Trp Val Asn Gln Phe Tyr Thr Leu Ala Pro
        515                 520                 525

Met Gly Asn Gly Ala Gly Arg Arg Arg Ala Leu
    530                 535


<210> 6
<211> 548
<212> PRT
<213> Hu/NLV/Sanbu 809/1998/JP

<400> 6
Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
  1               5                  10                  15

Gly Leu Val Pro Glu Ile Asn Asn Glu Ala Met Ala Leu Asp Pro Val
            20                  25                  30

Ala Gly Ala Ala Ile Ala Ala Pro Leu Thr Gly Gln Gln Asn Ile Ile
        35                  40                  45

Asp Pro Trp Ile Met Asn Asn Phe Val Gln Ala Pro Gly Gly Glu Phe
    50                  55                  60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Val Leu Leu Asn Leu Glu
65                  70                  75                  80

Leu Gly Pro Glu Ile Asn Pro Tyr Leu Ala His Leu Ala Arg Met Tyr
                85                  90                  95

Asn Gly Tyr Ala Gly Gly Phe Glu Val Gln Val Val Leu Ala Gly Asn
            100                 105                 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Ile Pro Pro Asn Phe
```

115 120 125

Pro Ile Asp Asn Leu Ser Ala Ala Gln Ile Thr Met Cys Pro His Val
130 135 140

Ile Val Asp Val Arg Gln Leu Glu Pro Val Asn Leu Pro Met Pro Asp
145 150 155 160

Val Arg Asn Asn Phe Phe His Tyr Asn Gln Gly Ser Asp Ser Arg Leu
165 170 175

Arg Leu Ile Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Asn Ser Gly
180 185 190

Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ser Pro
195 200 205

Asp Phe Ser Phe Asn Phe Leu Val Pro Pro Thr Val Glu Ser Lys Thr
210 215 220

Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Ser Glu Met Ser Asn Ser
225 230 235 240

Arg Phe Pro Val Pro Ile Glu Ser Leu His Thr Ser Pro Thr Glu Asn
245 250 255

Ile Val Val Gln Cys Gln Asn Gly Arg Val Thr Leu Asp Gly Glu Leu
260 265 270

Met Gly Thr Thr Gln Leu Leu Pro Ser Gln Ile Cys Ala Phe Arg Gly
275 280 285

Val Leu Thr Arg Ser Thr Ser Arg Ala Ser Asp Gln Ala Asp Thr Ala
290 295 300

Thr Pro Arg Leu Phe Asn Tyr Tyr Trp His Val Gln Leu Asp Asn Leu
305 310 315 320

Asn Gly Thr Pro Tyr Asp Pro Ala Glu Asp Ile Pro Gly Pro Leu Gly
325 330 335

Thr Pro Asp Phe Arg Gly Lys Val Phe Gly Val Ala Ser Gln Arg Asn
340 345 350

Leu Asp Ser Thr Thr Arg Ala His Glu Ala Lys Val Asp Thr Thr Ala
355 360 365

```
Gly Arg Phe Thr Pro Lys Leu Gly Ser Leu Glu Ile Ser Thr Asp Ser
    370                 375                 380

Asp Asp Phe Asp Gln Asn Gln Pro Thr Lys Phe Thr Pro Val Gly Ile
385                 390                 395                 400

Gly Val Asp Asn Glu Ala Glu Phe Gln Gln Trp Ser Leu Pro Asp Tyr
                405                 410                 415

Ser Gly Gln Phe Thr His Asn Met Asn Leu Ala Pro Ala Val Ala Pro
            420                 425                 430

Asn Phe Pro Gly Glu Gln Leu Leu Phe Phe Arg Ser Gln Leu Pro Ser
        435                 440                 445

Ser Gly Gly Arg Ser Asn Gly Val Leu Asp Cys Leu Val Pro Gln Glu
    450                 455                 460

Trp Val Gln His Phe Tyr Gln Glu Ser Ala Pro Ala Gln Thr Gln Val
465                 470                 475                 480

Ala Leu Val Arg Tyr Val Asn Pro Asp Thr Gly Lys Val Leu Phe Glu
                485                 490                 495

Ala Lys Leu His Lys Leu Gly Phe Met Thr Ile Ala Asn Asn Gly Asp
            500                 505                 510

Ser Pro Ile Thr Val Pro Pro Asn Gly Tyr Phe Arg Phe Glu Ser Trp
        515                 520                 525

Val Asn Pro Phe Tyr Thr Leu Ala Pro Met Gly Thr Gly Asn Gly Arg
    530                 535                 540

Arg Arg Ile Gln
545
```

<210> 7
<211> 540
<212> PRT
<213> Hu/NLV/Ichikawa 754/1998/JP

<400> 7

```
Met Lys Met Ala Ser Asn Asp Ala Thr Pro Ser Asn Asp Gly Ala Ala
  1               5                  10                  15
```

Gly Leu Val Pro Glu Ser Asn Asn Glu Ala Met Ala Leu Glu Pro Val
20 25 30

Val Gly Ala Ser Leu Ala Ala Pro Val Thr Gly Gln Thr Asn Ile Ile
35 40 45

Asp Pro Trp Ile Arg Thr Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
50 55 60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Ile Leu Val Asn Leu Glu
65 70 75 80

Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ala His Leu Ala Arg Met Tyr
85 90 95

Asn Gly Tyr Ala Gly Gly Met Glu Val Gln Val Met Leu Ala Gly Asn
100 105 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Val Pro Pro Tyr Phe
115 120 125

Pro Val Glu Asn Leu Ser Pro Ser Gln Ile Thr Met Phe Pro His Val
130 135 140

Ile Ile Asp Val Arg Thr Leu Glu Pro Val Leu Leu Pro Met Pro Asp
145 150 155 160

Val Arg Ser Thr Leu Phe His Phe Asn Gln Lys Asp Glu Pro Lys Met
165 170 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
180 185 190

Asp Asp Val Phe Thr Val Ser Cys Arg Ile Leu Thr Arg Pro Ser Pro
195 200 205

Glu Phe Asp Phe Thr Tyr Leu Val Pro Pro Thr Val Glu Ser Lys Thr
210 215 220

Lys Pro Phe Thr Leu Pro Val Leu Thr Leu Gly Glu Leu Ser Asn Ser
225 230 235 240

Arg Phe Pro Leu Ser Ile Asp Glu Met Val Thr Ser Pro Asn Glu Ser
245 250 255

Ile Val Val Gln Pro Gln Asn Gly Arg Val Thr Leu Asp Gly Glu Leu
260 265 270

Leu Gly Thr Thr Gln Leu Gln Ala Cys Asn Ile Cys Ser Ile Arg Gly
275 280 285

Lys Val Thr Gly Gln Val Pro Ser Glu Gln His Met Trp Asn Leu Glu
290 295 300

Ile Thr Asn Leu Asn Gly Thr Gln Phe Asp Pro Thr Asp Asp Val Pro
305 310 315 320

Ala Pro Leu Gly Val Pro Asp Phe Ala Gly Glu Val Phe Gly Val Leu
325 330 335

Ser Gln Arg Asn Arg Gly Glu Ser Asn Pro Ala Asn Arg Ala His Asp
340 345 350

Ala Val Val Ala Thr Tyr Ser Asp Lys Tyr Thr Pro Lys Leu Gly Leu
355 360 365

Val Gln Ile Gly Thr Trp Asn Thr Asn Asp Val Glu Asn Gln Pro Thr
370 375 380

Lys Phe Thr Pro Ile Gly Leu Asn Glu Val Ala Asn Gly His Arg Phe
385 390 395 400

Glu Gln Trp Thr Leu Pro Arg Tyr Ser Gly Ala Leu Thr Leu Asn Met
405 410 415

Asn Leu Ala Pro Ala Val Ala Pro Leu Phe Pro Gly Glu Arg Leu Leu
420 425 430

Phe Phe Arg Ser Tyr Val Pro Leu Lys Gly Gly Phe Gly Asn Pro Ala
435 440 445

Ile Asp Cys Ser Val Pro Gln Glu Trp Val Gln His Phe Tyr Gln Glu
450 455 460

Ser Ala Pro Ser Leu Gly Asp Val Ala Leu Val Arg Tyr Val Asn Pro
465 470 475 480

Asp Thr Gly Arg Val Leu Phe Glu Ala Lys Leu His Lys Gly Gly Phe
485 490 495

Leu Thr Val Ser Ser Thr Ser Thr Gly Pro Val Val Val Pro Ala Asn

500 505 510

Gly Tyr Phe Lys Phe Asp Ser Trp Val Asn Gln Phe Tyr Ser Leu Ala
515 520 525

Pro Met Gly Thr Gly Asn Gly Arg Arg Arg Val Gln
530 535 540

<210> 8
<211> 535
<212> PRT
<213> Hu/NLV/Chitta 1876/1996/JP

<400> 8
Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
1 5 10 15

Gly Leu Val Pro Glu Ala Asn Asn Glu Thr Met Ala Leu Glu Pro Val
20 25 30

Ala Gly Ala Ser Ile Ala Ala Pro Leu Thr Gly Gln Asn Asn Ile Ile
35 40 45

Asp Pro Trp Ile Arg Leu Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
50 55 60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Val Leu Leu Asn Leu Glu
65 70 75 80

Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ala His Leu Ser Arg Met Tyr
85 90 95

Asn Gly Tyr Ala Gly Gly Val Glu Val Gln Val Leu Leu Ala Gly Asn
100 105 110

Ala Phe Thr Ala Gly Lys Leu Val Phe Ala Ala Val Pro Pro His Phe
115 120 125

Pro Leu Glu Asn Ile Ser Pro Gly Gln Ile Thr Met Phe Pro His Val
130 135 140

Ile Ile Asp Val Arg Thr Leu Glu Pro Val Leu Leu Pro Leu Pro Asp
145 150 155 160

Val Arg Asn Asn Phe Phe His Tyr Asn Gln Gln Asn Glu Pro Arg Met

```
                165                 170                 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
            180                 185                 190

Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ser Pro
        195                 200                 205

Asp Phe Asp Phe Asn Tyr Leu Val Pro Pro Thr Leu Glu Ser Lys Thr
    210                 215                 220

Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Gly Glu Leu Thr Asn Ser
225                 230                 235                 240

Arg Phe Pro Val Pro Ile Asp Glu Leu Tyr Thr Ser Pro Asn Glu Ser
                245                 250                 255

Leu Val Val Gln Pro Gln Asn Gly Arg Cys Ala Leu Asp Gly Glu Leu
            260                 265                 270

Gln Gly Thr Thr Gln Leu Leu Pro Thr Ala Ile Cys Ser Phe Arg Gly
        275                 280                 285

Arg Ile Asn Gln Lys Val Ser Gly Glu Asn His Val Trp Asn Met Gln
    290                 295                 300

Val Thr Asn Ile Asn Gly Thr Pro Phe Asp Pro Thr Gly Asp Val Pro
305                 310                 315                 320

Ala Pro Leu Gly Thr Pro Asp Phe Ser Gly Lys Leu Phe Gly Val Leu
                325                 330                 335

Ser Gln Arg Asp His Asp Asn Ala Cys Arg Ser His Asp Ala Val Ile
            340                 345                 350

Ala Thr Asn Ser Ala Lys Phe Thr Pro Lys Leu Gly Ala Ile Gln Ile
        355                 360                 365

Gly Thr Trp Glu Glu Asp Asp Val His Ile Asn Gln Pro Thr Lys Phe
    370                 375                 380

Thr Pro Val Gly Leu Phe Glu Asn Glu Gly Phe Asn Gln Trp Thr Leu
385                 390                 395                 400

Pro Asn Tyr Ser Gly Ala Leu Thr Leu Asn Met Gly Leu Ala Pro Pro
                405                 410                 415
```

Val Ala Pro Thr Phe Pro Gly Glu Gln Ile Leu Phe Phe Arg Ser His
420 425 430

Ile Pro Leu Lys Gly Gly Val Ala Asp Pro Val Ile Asp Cys Leu Leu
435 440 445

Pro Gln Glu Trp Ile Gln His Leu Tyr Gln Glu Ser Ala Pro Ser Gln
450 455 460

Ser Asp Val Ala Leu Ile Arg Phe Thr Asn Pro Asp Thr Gly Arg Val
465 470 475 480

Leu Phe Glu Ala Lys Leu His Arg Ser Gly Tyr Ile Thr Val Ala Asn
485 490 495

Thr Gly Ser Arg Pro Ile Val Val Pro Ala Asn Gly Tyr Phe Arg Phe
500 505 510

Asp Thr Trp Val Asn Gln Phe Tyr Ser Leu Ala Pro Met Gly Thr Gly
515 520 525

Asn Gly Arg Arg Arg Val Gln
530 535

<210> 9
<211> 542
<212> PRT
<213> Hu/NLV/Kashiwa 47/1997/JP

<400> 9
Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
1 5 10 15

Ser Leu Val Pro Glu Gly Ile Asn Glu Thr Met Pro Leu Glu Pro Val
20 25 30

Ala Gly Ala Ser Ile Ala Ala Pro Val Ala Gly Gln Thr Asn Ile Ile
35 40 45

Asp Pro Trp Ile Arg Thr Asn Phe Val Gln Ala Pro Asn Gly Glu Phe
50 55 60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Ile Leu Leu Asn Leu Glu
65 70 75 80

Leu Gly Pro Asp Leu Asn Pro Tyr Leu Ala His Leu Ser Arg Met Tyr
85 90 95

Asn Gly Tyr Ala Gly Gly Val Glu Val Gln Val Leu Leu Ala Gly Asn
100 105 110

Ala Phe Thr Ala Gly Lys Ile Leu Phe Ala Ala Ile Pro Pro Asn Phe
115 120 125

Leu Val Asp Met Ile Ser Pro Ala Gln Ile Thr Met Leu Pro His Leu
130 135 140

Ile Val Asp Val Arg Thr Leu Glu Pro Ile Met Thr Pro Leu Pro Asp
145 150 155 160

Val Arg Asn Val Phe Tyr His Phe Asn Asn Gln Pro Gln Pro Arg Met
165 170 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ser Asn Gly Ser Gly
180 185 190

Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Thr Pro
195 200 205

Asp Phe Glu Phe Ile Tyr Leu Val Pro Pro Ser Val Glu Ser Lys Thr
210 215 220

Lys Pro Phe Thr Leu Pro Ile Leu Thr Ile Ser Glu Leu Thr Asn Ser
225 230 235 240

Arg Phe Pro Ile Pro Ile Glu Gln Leu Tyr Thr Ala Pro Asn Glu Thr
245 250 255

Asn Val Val Gln Cys Gln Asn Gly Arg Cys Thr Leu Asp Gly Glu Leu
260 265 270

Gln Gly Thr Thr Gln Leu Leu Ser Ser Ala Val Cys Phe Leu Gln Gly
275 280 285

Arg Thr Val Ala Asp Asn Gly Asp Asn Trp Asp Gln Asn Leu Leu Gln
290 295 300

Leu Thr Tyr Pro Asn Gly Ala Ser Tyr Asp Pro Thr Asp Glu Val Pro
305 310 315 320

Ala Pro Leu Gly Thr Gln Asp Phe Ser Gly Met Leu Tyr Gly Val Leu
325 330 335

Thr Gln Asp Asn Val Asn Val Ser Thr Gly Glu Ala Lys Asn Ala Lys
340 345 350

Gly Ile Tyr Ile Ser Thr Thr Ser Gly Lys Phe Thr Pro Lys Ile Gly
355 360 365

Ser Ile Gly Leu His Ser Ile Thr Glu His Val His Pro Asn Gln Gln
370 375 380

Ser Arg Phe Thr Pro Val Gly Val Ala Val Asp Glu Asn Thr Pro Phe
385 390 395 400

Gln Gln Trp Val Leu Pro His Tyr Ala Gly Ser Leu Ala Leu Asn Thr
405 410 415

Asn Leu Ala Pro Ala Val Ala Pro Thr Phe Pro Gly Glu Gln Leu Leu
420 425 430

Phe Phe Arg Ser Arg Val Pro Cys Val Gln Gly Leu Gln Gly Gln Asp
435 440 445

Ala Phe Ile Asp Cys Leu Leu Pro Gln Glu Trp Val Asn His Phe Tyr
450 455 460

Gln Glu Ala Ala Pro Ser Gln Ala Asp Val Ala Leu Ile Arg Tyr Val
465 470 475 480

Asn Pro Asp Thr Gly Arg Thr Leu Phe Glu Ala Lys Leu His Arg Ser
485 490 495

Gly Phe Ile Thr Val Ser His Thr Gly Ala Tyr Pro Leu Val Val Pro
500 505 510

Pro Asn Gly His Phe Arg Phe Asp Ser Trp Val Asn Gln Phe Tyr Ser
515 520 525

Leu Ala Pro Met Gly Thr Gly Asn Gly Arg Arg Arg Ile Gln
530 535 540

<210> 10
<211> 550
<212> PRT

<213> Hu/NLV/Mie 7k/1994/JP

<400> 10

```
Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Asn Asp Gly Ala Ala
 1               5                  10                  15

Asn Leu Val Pro Glu Ala Asn Asp Glu Val Met Ala Leu Glu Pro Val
            20                  25                  30

Val Gly Ala Ser Ile Ala Ala Pro Val Val Gly Gln Gln Asn Ile Ile
        35                  40                  45

Asp Pro Trp Ile Arg Glu Asn Phe Val Gln Ala Pro Gln Gly Glu Phe
    50                  55                  60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Met Leu Leu Asn Leu Glu
65                  70                  75                  80

Leu Gly Pro Glu Leu Asn Pro Tyr Leu Ser His Leu Ser Arg Met Tyr
                85                  90                  95

Asn Gly Tyr Ala Gly Gly Met Gln Val Gln Val Val Leu Ala Gly Asn
            100                 105                 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Val Pro Pro His Phe
        115                 120                 125

Pro Val Glu Asn Ile Ser Ala Ala Gln Ile Thr Met Cys Pro His Val
    130                 135                 140

Ile Val Asp Val Arg Gln Leu Glu Pro Val Leu Leu Pro Leu Pro Asp
145                 150                 155                 160

Ile Arg Asn Arg Phe Phe His Tyr Asn Gln Glu Asn Thr Pro Arg Met
                165                 170                 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Ser Gly Glu
            180                 185                 190

Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Arg Pro Ala Pro Asp
        195                 200                 205

Phe Glu Phe Thr Phe Leu Val Pro Pro Thr Val Glu Ser Lys Thr Lys
    210                 215                 220

Pro Phe Thr Leu Pro Ile Leu Thr Leu Gly Glu Leu Ser Asn Ser Arg
```

225 230 235 240

Phe Pro Ala Ala Ile Asp Met Leu Tyr Thr Asp Pro Asn Glu Ser Ile
245 250 255

Val Val Gln Pro Gln Asn Gly Arg Cys Thr Leu Asp Gly Thr Leu Gln
260 265 270

Gly Thr Thr Gln Leu Val Pro Thr Gln Ile Cys Ala Phe Arg Gly Thr
275 280 285

Leu Ile Ser Gln Thr Ala Arg Ala Ala Asp Ser Thr Asp Ser Pro Gln
290 295 300

Arg Ala Arg Asn His Pro Leu His Val Gln Val Lys Asn Leu Asp Gly
305 310 315 320

Thr Gln Tyr Asp Pro Thr Asp Asp Ile Pro Ala Val Leu Gly Ala Ile
325 330 335

Asp Phe Lys Gly Thr Val Phe Gly Val Ala Ser Gln Arg Asp Val Ser
340 345 350

Gly Gln Gln Glu Gln Gly His Tyr Ala Thr Arg Ala His Glu Ala His
355 360 365

Ile Asp Thr Thr Asp Pro Lys Tyr Ala Pro Lys Leu Gly Thr Ile Leu
370 375 380

Ile Lys Ser Gly Ser Asp Asp Phe Asn Thr Asn Gln Pro Ile Arg Phe
385 390 395 400

Thr Pro Val Gly Met Gly Asp Asn Asn Trp Arg Gln Trp Glu Leu Pro
405 410 415

Asp Tyr Ser Gly Arg Leu Thr Leu Asn Met Asn Leu Ala Pro Ala Val
420 425 430

Ser Pro Ser Phe Pro Gly Glu Arg Ile Leu Phe Phe Arg Ser Ile Val
435 440 445

Pro Ser Ala Gly Gly Tyr Gly Ser Gly Tyr Ile Asp Cys Leu Ile Pro
450 455 460

Gln Glu Trp Val Gln His Phe Tyr Gln Glu Ala Ala Pro Ser Gln Ser
465 470 475 480

Ala Val Ala Leu Val Arg Tyr Val Asn Pro Asp Thr Gly Arg Asn Ile
485 490 495

Phe Glu Ala Lys Leu His Arg Glu Gly Phe Leu Thr Val Ala Asn Cys
500 505 510

Gly Asn Asn Pro Ile Val Val Pro Pro Asn Gly Tyr Phe Arg Phe Glu
515 520 525

Ala Trp Gly Asn Gln Phe Tyr Thr Leu Ala Pro Met Gly Ser Gly Gln
530 535 540

Gly Arg Arg Arg Ala Gln
545 550

<210> 11
<211> 541
<212> PRT
<213> Hu/NLV/Osaka 10-25/1999/JP

<400> 11
Met Lys Met Ala Ser Asn Asp Ala Ala Pro Ser Ser Asp Gly Ala Ala
1 5 10 15

Gly Leu Val Pro Glu Ile Asn Asn Glu Val Met Pro Leu Glu Pro Val
20 25 30

Ala Gly Ala Ser Leu Ala Thr Pro Val Val Gly Gln Gln Asn Ile Ile
35 40 45

Asp Pro Trp Ile Arg Asn Asn Phe Val Gln Ala Pro Ala Gly Glu Phe
50 55 60

Thr Val Ser Pro Arg Asn Ser Pro Gly Glu Ile Leu Leu Asp Leu Glu
65 70 75 80

Leu Gly Pro Asp Leu Asn Pro Tyr Leu Ala His Leu Ala Arg Met Tyr
85 90 95

Asn Gly His Ala Gly Gly Met Glu Val Gln Ile Val Leu Ala Gly Asn
100 105 110

Ala Phe Thr Ala Gly Lys Ile Ile Phe Ala Ala Ile Pro Pro Gly Phe
115 120 125

```
Pro Tyr Glu Asn Leu Ser Pro Ser Gln Ile Thr Met Cys Pro His Val
    130                 135                 140

Ile Ile Asp Val Arg Gln Leu Glu Pro Phe Leu Leu Pro Met Pro Asp
145                 150                 155                 160

Ile Trp Asn Asn Phe Phe His Tyr Asn Gln Gly Asn Asp Pro Lys Leu
                165                 170                 175

Arg Leu Val Ala Met Leu Tyr Thr Pro Leu Arg Ala Asn Asn Ser Gly
            180                 185                 190

Asp Asp Val Phe Thr Val Ser Cys Arg Val Leu Thr Lys Pro Ser Pro
        195                 200                 205

Asp Phe Glu Phe Thr Phe Leu Val Pro Pro Thr Val Glu Ser Lys Thr
    210                 215                 220

Lys Gln Phe Ala Leu Pro Ile Leu Lys Ile Ser Glu Met Thr Asn Ser
225                 230                 235                 240

Arg Phe Pro Val Pro Val Asp Val Met Tyr Thr Ala Arg Asn Glu Asn
                245                 250                 255

Gln Val Val Gln Pro Gln Asn Gly Arg Val Thr Leu Asp Gly Glu Leu
            260                 265                 270

Leu Gly Thr Thr Pro Leu Leu Ala Val Asn Ile Cys Lys Phe Lys Gly
        275                 280                 285

Glu Val Ile Ala Lys Asn Gly Asp Val Arg Ser Tyr Arg Met Asp Met
    290                 295                 300

Glu Ile Thr Asn Thr Asp Gly Thr Pro Ile Asp Pro Thr Glu Asp Thr
305                 310                 315                 320

Pro Gly Pro Ile Gly Ser Pro Asp Phe Gln Gly Ile Leu Phe Gly Val
                325                 330                 335

Ala Ser Gln Arg Asn Lys Asn Glu Gln Asn Pro Ala Thr Arg Ala His
            340                 345                 350

Glu Ala Ile Ile Asn Thr Gly Gly Asp His Leu Cys Pro Gln Ile Ser
        355                 360                 365
```

Ser Ser Glu Ile Tyr Leu Thr Ser Pro Asn Ile Leu Arg Cys Thr Asn
370 375 380

Pro Gln Pro Leu Pro Gln Ser Gly Leu Arg Gly Thr Ile Leu Ile Arg
385 390 395 400

Ser Asp Asn Gly His Cys His Asp Met Val Gly Thr Ser Pro Thr Thr
405 410 415

Pro Thr Trp Pro Gln Gln Trp Arg Arg Cys Ser Arg Gly Ser Asn Cys
420 425 430

Cys Ser Ser Gly His Arg Tyr Pro Val Pro Val Val Met Asn Arg Val
435 440 445

Thr Trp Ile Val Leu Ser His Lys Ser Gly Phe Ser Thr Ser Thr Arg
450 455 460

Lys Leu Pro Gln Leu Asn Leu Arg Trp Pro Leu Ile Arg Phe Ile Asn
465 470 475 480

Pro Asp Thr Gly Arg Val Leu Phe Glu Ala Arg Leu His Lys Gln Gly
485 490 495

Phe Ile Thr Val Ala His Thr Gly Asp Asn Pro Ile Val Met Pro Pro
500 505 510

Asn Gly Tyr Phe Arg Phe Glu Ala Trp Val Asn Gln Phe Tyr Ser Leu
515 520 525

Ala Pro Val Gly Thr Gly Lys Gly Arg Arg Arg Val Gln
530 535 540

<210> 12
<211> 1638
<212> DNA
<213> Hu/NLV/Kashiwa 645/1999/JP

<400> 12
atgatgatgg cgtctaagga cgccccaaca aacatggatg gcaccagtgg tgccggccag 60
ctggtaccag aggcaaatac agctgagcct atatcaatgg agcctgtggc tggggcagca 120
acagctgccg caaccgctgg ccaagttaat atgattgacc cctggataat gaataattat 180
gtgcaagccc ctcaaggtga atttaccata tcgcctaata acacaccagg tgatattttg 240
tttgatctac aattaggccc tcatctcaat cctttcttat cccatttggc ccaaatgtat 300
aacggttggg ttggcaatat gaaagtgaag gtcctattgg ctggtaatgc cttcacggct 360

ggtaaaataa tcattagttg catacccccct ggctttgctg cgcaaaacat ttctatcgct 420
caggccacaa tgttccccca cgttatagct gatgttaggg ttttggaacc tattgaggtg 480
ccattggaag atgtgaggaa tgtgctgttc cataacaatg acaacgcacc aaccatgagg 540
ttggtgtgca tgctctacac cccccttgcga gccagtggta gctcatctgg aactgaccct 600
tttgtgattg ctgggcgtgt tctgacatgc ccaagccctg actttagctt cttattcttg 660
gttcccccca atgtagagca aaagactaaa cctttagtg tcccaaatct tccactgaat 720
acccttttcaa attcaagagt cccttctcta attaaatcaa tgatggtatc cagagaccat 780
gggcagatgg ttcagtttca aaacggtagg gtcaccctgg atgggcaact gcaaggcacc 840
acgcccacat cagctagcca gctgtgcaaa atcagaggca gtgtcttcca tgctaatggt 900
gggaatggat ataacctaac tgaattggat gggagcccat accatgcttt tgagagccct 960
gcgccaatag ggtttcctga tctaggtgaa tgtgattggc acatggaggc ctcccctacc 1020
acccaattca atactggtga tgttataaaa caaattaatg tcaaacaaga atcagcattt 1080
gctcccccacc ttggtaccat acaagcagat ggcctgagtg atgtgagtgt caacactaac 1140
atgatagcca aattgggatg ggtgtcaccc gtcagtgatg gacatagagg agatgtcgat 1200
ccgtgggtca ttccacgata tgggtcgact ttgaccgagg ccgcccaatt agcccccccca 1260
atatatcccc caggttttgg tgaggccatt gtgtttttca tgtcagattt tcctatagcc 1320
catggtacca atggcttgag tgtgccttgc accatacccc aagaatttgt cacccatttt 1380
gtcaatgaac aggcccctac tagaggggaa gcagccctac tgcattattt agaccctgat 1440
acccatagaa atcttggtga gtttaaatta taccctgagg ggttcatgac gtgtgtgcct 1500
aattccagtg gcactggtcc acaaaccctc ccaatcaatg gtgtttttgt ttttgtgtcc 1560
tgggtttcca gattctatca gttaaagcct gtgggaacag ccggcccggc ttgtaggctt 1620
ggcatcagaa gatcataa 1638

<210> 13
<211> 1593
<212> DNA
<213> Hu/NLV/Seto 124/1989/JP

<400> 13
atgatgatgg cgtctaagga cgctacgtca agcgtggatg gcgccagtgg cgctggtcag 60
ttggtaccgg aggttaatgc ttctgaccct cttgcaatgg atcctgtggc gggttcttca 120
acagcagttg caactgctgg gcaagttaac cctattgacc cttggataat caataacttt 180
gtgcaggctc cccaaggtga atttactatt tctccaaata atacccccgg tggtgttttg 240
tttgatttga gtctaggccc tcatcttaat cccttcttgt tacatttgtc acaaatgtat 300
aatggctggg ttggcaacat gagagttagg attatgctgg ctggtaatgc atttactgca 360
ggcaaaatta tagtttcttg catacctcct ggctttggct cccataatct tactatagca 420
caagcaactc tcttcccgca tgtgattgct gatgttagga ctttagaccc aattgaagta 480
cccttggaag atgtaaggaa tgttctcttt cataataatg atagaaatca acaaaccatg 540
cgccttgtgt gtatgcttta tacccccctc cgcactggtg gcggtacagg tgattctttt 600
gtggttgcag ggcgagtcat gacttgtcct agccccgatt tcaatttctt gttcttggtt 660
cctcccacag tggaacagaa gactaggcct ttcaccctcc caaatttacc gctgagttct 720
ttgtcaaatt cacgtgctcc tcttccaatt agtggcatgg gtatttctcc agacaatgtt 780
cagagtgtgc agtttcaaaa tggccgatgt accttagacg ggcgtcttgt tggtaccacc 840
ccagtttccc tctcccacgt tgctaagata aggggcactt ctaatggtac tgtgatcaat 900
ctcaccgaat tggatggcac ccccttccac cctttgaag gccctgcccc tattggtttt 960

ccagatcttg gtggctgtga ttggcatatt aatatgacac aatttgggca ttccagtcag 1020
actcaatatg atgtagatac cacccccgac accttcgtcc ctcacttagg ttcaatccag 1080
gcgaatggca ttggtagtgg caactatatt ggtgttctta gctgggtctc ccccccatca 1140
catccatctg gctctcaagt tgatctctgg aagatcccca actatgggtc tagcatcaca 1200
gaggcaaccc atctagctcc ctctgtctat cctcctggct ttggagaggt gttagtcttt 1260
ttcatgtcaa agatacctgg tcctggtgct tatagtctgc cctgtttact gccacaagaa 1320
tatatctcac acctcgcaag tgaacaagcc cccactgttg gtgaggccgc cttgctccac 1380
tatgttgacc ctgacacggg ccggactctt ggggagttta aggcttaccc tgatggtttc 1440
ctaacctgtg tccctaacgg ggccagctcg ggcccacaac aactaccaat caatggagtc 1500
tttgtctttg tttcatgggt gtccagattt tatcagttaa agcctgtggg aactgccagt 1560
tcggcaagag gtaggcttgg tttgcgccga taa 1593

<210> 14
<211> 1641
<212> DNA
<213> Hu/NLV/Funabashi 258/1996/JP

<400> 14
atgatgatgg cgtctaagga cgcccctcaa agcgctgatg gcgcaagcgg cgcaggtcaa 60
ctggtgccgg aggttaatac agctgacccc ttacccatgg aacccgtggc tgggccaaca 120
acagccgtag ccactgctgg gcaagttaat atgattgatc cctggattgt taataatttt 180
gtccagtcac cacaaggtga gtttacaatt tcccctaata ataccccccgg tgatattttg 240
tttgatttac aattaggtcc acatctaaac cctttcttgt cacatctgtc ccaaatgtat 300
aatggctggg ttggaaacat gagagttagg attctccttg ctgggaatgc attctcagct 360
ggaaagatta tagtttgttg tgtcccccct ggctttacat cttcctctct caccatagct 420
caggctacat tgtttcccca tgtgattgct gatgtgagaa cccttgaacc aatagaaatg 480
cccctcgagg atgtacgcaa tgtcctctat cacaccaatg ataatcaacc aacaatgcgg 540
ttggtgtgta tgctgtacac gccgctccgc actggtgggg ggtctggtaa ttctgattct 600
tttgtggttg ctggcagggt gctcacggcc cctagtagcg acttcagttt cttgttcctt 660
gtcccgccta ccatagaaca gaagactcgg gcttttactg tgcctaatat ccccttgcaa 720
accttgtcca attctaggtt tccttccctc atccagggga tgattctgtc tcctgacgca 780
tctcaagtgg tccaattcca aaatggacgt tgcctcatag atggtcaact cctaggcact 840
acacccgcta catcaggaca gctgttcaga gtaagaggaa agataaatca gggagcccgt 900
acgctcaacc tcacagaggt ggatggcaaa ccattcatgg catttgattc ccctgcacct 960
gtggggttcc ccgattttgg aaaatgtgat tggcacatga gaatcagcaa aaccccaaat 1020
aacacaagct caggtgaccc catgcgcagt gtcagcgtgc aaaccaatgt gcagggtttt 1080
gtgccacacc taggaagtat acagtttgat gaagtgttca accaccccac aggtgactac 1140
attggcacca ttgaatggat ttcccagcca tctacacccc ctggaacaga tattaatctg 1200
tgggagattc ccgattatgg atcatccctt tcccaagcag ctaatctggc ccccccagta 1260
ttcccccctg gatttggtga ggctcttgtg tactttgttt ctgcttttcc aggccccaac 1320
aaccgctcag ccccgaatga tgtaccttgt cttctccctc aagagtacat aacccacttt 1380
gtcagtgaac aagccccaac gatgggtgac gcagctttgc tgcattatgt cgaccctgat 1440
accaacagaa accttgggga gttcaagcta taccctggag gttacctcac ctgtgtacca 1500
aacggggtgg gtgccgggcc tcaacagctt cctcttaatg gtgtctttct ctttgtctct 1560
tgggtgtctc gttttatca gctcaagcct gtgggaacag ccagtacggc aagaagtagg 1620

cttggagtgc gccgtatata a 1641

<210> 15
<211> 1635
<212> DNA
<213> Hu/NLV/Chiba 407/1987/JP

<400> 15
atgatgatgg cgtctaagga cgctacacca agcgcagatg gcgccactgg cgccggccag 60
ctggtaccgg aggttaatac agctgacccc atacctattg accctgtggc tggctcctct 120
acagcccttg ccacagcagg ccaggttaat ttgattgatc cctggataat caataatttt 180
gtgcaagccc cccagggcga gttcacaata tccccaaata atacccccgg tgatgtgctt 240
tttgatttgc aattaggacc ccatttaaat cctttccttt cccacctttc tcagatgtat 300
aatggttggg tgggcaacat gcgagtgcgt gttgtcttgg ctggtaatgc tttcacggct 360
gggaaggtta taatttgttg tgtccccccct ggtttccaat ctcgcaccct ttctatagcc 420
caggctactt tatttcccca tgtaattgct gatgttagga cccttgaccc tgtagaagtg 480
ccccttgaag atgttaggaa tgtgttgtat cataataatg acacccaacc caccatgcgc 540
ctcctttgca tgttgtacac tcctctccgc accgggggag cgtctggtgg gactgattct 600
tttgtggtgg ctgggcgtgt actcacttgt ccgggccctg actttaactt cttattccta 660
gtccctccca cagtcgagca aaagacccgc ccttttactg tgcctaatat ccctttgaag 720
tacctgtcta attccaggat cccaaatcct attgaaggta tgtcattgtc acctgaccag 780
acccaaaatg ttcaattcca gaatggtagg tgtacaattg acggtcaacc ccttgggacc 840
acacctgtct cagttagtca gttatgtaag tttaggggta ggattacatc tggacagaga 900
gtgctcaact tgacagagtt ggatggttca ccttttatgg cctttgccgc ccccgcccct 960
gcgggctttc cagatcttgg gtcctgtgat tggcatattg aaatgagtaa aatcccaaat 1020
tccagcaccc agaacaaccc aatagtgacc aattctgtca aacccaatag tcaacagttt 1080
gtcccacact tgtcaagtat caccсttgat gaaaatgttt ccagtggagg tgactatatt 1140
ggcactatac aatggacctc acctccttct gattctggcg ggccaatac aaatttttgg 1200
aaaatccctg actatgggtc cagcctagca gaagcttcac aactggcccc cgctgtctat 1260
ccacctggtt tcaatgaggt gattgtgtat tttatggcat ctatacctgg tcccaatcag 1320
tctgggtctc ctaatttagt gccatgcctg ctcccccagg aatatataac acactttatt 1380
agtgagcagg cccccatcca gggtgaggct gccttactcc actatgtaga cccagacacc 1440
aatcgcaatt tgggtgagtt caaattatat cctggtggtt atttaacctg tgttcctaat 1500
agttctagta ctggacctca acaacttcct cttgatggtg tatttgtctt tgcttcttgg 1560
gtttctagat tttatcaatt aaagcctgtg ggaacagccg gaccggctag aggtaggctt 1620
ggtgtccgta gataa 1635

<210> 16
<211> 1620
<212> DNA
<213> Hu/NLV/Narita 104/1997/JP

<400> 16
atgaagatgg cgtcgaatga cgccaaccca tctgatgggt ccacagccaa cctcgtccca 60

gaggtcaaca atgaggttat ggctttggag cccgttgttg gtgccgctat tgcggcacct 120
gtagcgggcc aacaaaatgt aattgacccc tggattagaa ataattttgt acaagccccc 180
ggtggagagt ttacagtatc ccctagaaac gctccgggtg agatattatg gagcgcgccc 240
ttgggccctg atttgaaccc ctaccttttct catttggcca gaatgtacaa tggttatgca 300
ggtggttttg aagtgcaggt aatcctcgcg gggaacgcgt tcaccgccgg gaaaatcata 360
tttgcagcag tcccaccaaa ttttccaact gaaggcttga gccccagcca ggttactatg 420
ttccccccata taatagtaga tgttaggcaa ttggaacctg tattgatccc cttacctgat 480
gttaggaata acttctatca ttacaatcaa tcaaatgatt ctaccattaa attgatagca 540
atgctgtata caccacttag ggctaataat gctggggatg atgtcttcac agtctcttgt 600
cgagtcctca cgaggccatc ccccgatttt gatttcatat tcttggtgcc acccacagtt 660
gaatcaagaa ctaaaccatt caccgtccca atcttaactg ttgaggaaat gtctaactca 720
agattcccca ttcctttgga aaagttgtac acgggtccca gcagtgcttt tgttgtccaa 780
ccacaaaatg gcaggtgcac gactgatggc gtgctcttag gcactaccca gctgtctgct 840
gtcaacatct gcaccttcag aggggatgtc acccacattg caggcagtca tgactataca 900
atgaatttgg cttctcaaaa ttggagcaat tatgacccaa cagaagaaat cccagcccct 960
ctgggaactc cagatttcgt gggaaagatc caaggcatgc tcacccaaac cacaagagag 1020
gatggctcga cccgcgccca caaagctaca gtgagcactg ggagtgtcca cttcactcca 1080
aagctgggca gtgttcaata caccactgac acaaacaatg attttcaaac tggccaaaac 1140
acgaaattca ccccagtcgg cgtcatccag gacggtaata atcatcaaaa tgaaccccaa 1200
caatgggtgc tcccaaatta ctcaggtaga actggtcata atgtgcacct agctcctgcc 1260
gttgcccccа ctttcccggg tgagcaactt cttttcttta ggtccactat gcccggatgt 1320
agcgggtatc ctaacatgaa tctggattgc ctactccccc aggaatgggt gcaacacttc 1380
tgccaagaag cagctccagc acaatctgat gtggctctgc tgagatttgt gaatccagac 1440
acaggtaggg ttttgtttga gtgcaagctc cataaatcag gctatgtcac agtggctcac 1500
actggcccgc atgatttggt tatccccccc aatggttact ttagatttga ctcctgggtc 1560
aaccagttct acacacttgc ccccatggga aatggagcgg ggcgcaggcg tgcattataa 1620

<210> 17
<211> 1647
<212> DNA
<213> Hu/NLV/Sanbu 809/1998/JP

<400> 17
atgaagatgg cgtcgaatga cgctgctcca tctaatgatg gtgccgccgg cctcgtccca 60
gagatcaaca atgaggcaat ggcgctagac ccagtggcgg gtgcagcgat agcagcgccc 120
ctcactggtc agcaaaacat aattgatccc tggattatga ataattttgt gcaagcacct 180
ggtggtgagt ttacagtgtc ccctaggaat tcccctggtg aagtgcttct taatttggaa 240
ttgggcccag aaataaaccc ttatttggcc catcttgcta gaatgtataa tggttatgca 300
ggtggatttg aagtgcaggt ggtcctggct gggaatgcgt tcacagcagg aaagataatc 360
tttgcagcta tacccccctaa ttttccaatt gataatctga gcgcagcaca aatcactatg 420
tgcccgcatg tgattgtgga tgtcagacag ttggaaccgg tcaaccttcc gatgcctgac 480
gttcgcaaca atttctttca ttacaatcaa gggtctgatt cgcgattgcg cttaattgca 540
atgctgtata cacctcttag ggcaaataat tctggagatg atgttttcac tgtgtcttgt 600
agagtactga ctaggcctag ccctgatttt tcattcaatt tccttgtccc acccaccgtg 660
gaatcaaaga caaaaccctt taccctccct attctgacta tctctgaaat gtccaattct 720

aggtttccag tgccgattga gtctttgcac accagcccaa ctgagaatat tgttgtccag 780
tgccaaaatg ggcgcgtcac tctcgatggt gagttgatgg gcaccaccca actcttaccg 840
agtcaaattt gtgcttttag gggcgtgctc accagatcaa caagcagggc cagtgatcag 900
gccgatacag caacccctag gctgtttaat tattattggc atgtacaatt ggataatcta 960
aatgggaccc cttatgatcc tgcagaagac ataccaggcc ccctagggac accagacttc 1020
cggggcaagg tctttggcgt ggccagccag agaaacctcg acagcacaac tagagcacat 1080
gaagcaaaag tggacacaac agctggtcgt ttcaccccaa agttgggctc attagaaata 1140
tctactgatt ccgatgactt tgaccaaaac cagccaacaa agttcacccc agttggcatt 1200
ggggttgaca atgaggcaga atttcagcaa tggtctttac ccgactattc tggtcagttc 1260
acccacaaca tgaacctggc cccagctgtt gctcccaact tccctggtga gcagctcctt 1320
ttcttccgct cacagttacc atcttctggt gggcgatcca acggggtcct agactgtctg 1380
gtcccccagg aatgggtcca acacttctac caggaatcgg ccccccgccca aacacaagtg 1440
gccctggtta ggtatgtcaa ccctgacact ggtaaagtgc tatttgaggc caagctgcat 1500
aaattaggtt ttatgactat agctaacaat ggtgattctc caataactgt tcccccaaat 1560
ggatatttta ggtttgaatc ttgggtgaac ccctttttata cacttgcccc catgggaact 1620
gggaacgggc gtagaaggat tcaataa 1647

<210> 18
<211> 1623
<212> DNA
<213> Hu/NLV/Ichikawa 754/1998/JP

<400> 18
atgaagatgg cgtcgaatga cgctactcca tctaatgatg gtgccgccgg cctcgtgcca 60
gaaagtaaca atgaggcaat ggctctggaa cccgtggtgg gggcgtcttt agccgccccc 120
gtcactggcc aaactaatat aatagacccc tggattagaa ctaattttgt ccaagccccc 180
aatggtgaat ttacagtttc ccctagaaat tcccctggag agatattggt caatttggag 240
ttgggtccag aactgaaccc ttatctggca catttagcta ggatgtacaa tggttatgcg 300
ggtggtatgg aggtgcaagt gatgctcgcg gggaacgcgt tcactgctgg caagatcatc 360
tttgccgccg tgccacctta ctttccagtg gaaaatctta gcccttccca aataacaatg 420
ttcccacatg tgatcatcga tgtcagaacc ttggaacctg tattactccc aatgcctgat 480
gtcagaagca ccctttttcca ctttaatcaa aaagatgagc ctaagatgag acttgttgcc 540
atgctttaca ccccccttcg ttctaatggt tctggtgacg acgttttcac cgtctcatgt 600
aggatcctca ctaggccctc ccctgaattt gattttacat atttggtgcc accaacagta 660
gaatcaaaga ctaagccatt cacactacct gtgctgacac tgggagaact gtccaactct 720
agattccctc tctctattga tgaaatggtc accagcccca atgagtccat agttgttcag 780
ccacagaatg gtagggtcac actagatggg gagctgttag gcacaaccca actgcaagca 840
tgcaacattt gctccataag ggggaaggta acagggcagg tccctagtga acaacacatg 900
tggaacctgg agatcacaaa cctaaatggg acgcaatttg accctacaga tgatgtccca 960
gccccccttg gtgtgcccga ctttgcaggt gaggtctttg gtgtactcag ccagagaaat 1020
agaggtgaaa gcaacccagc aaacagggct catgacgctg tcgtggctac ctacagtgac 1080
aagtacaccc ctaaactagg cttagtgcaa attggaactt ggaacaccaa tgatgttgaa 1140
aaccagccaa caaaattcac cccaattggt ttgaatgagg tcgccaatgg ccatcgattt 1200
gaacagtgga ctttgcctag gtattctggt gccctgacat taaatatgaa tttagccccc 1260
gctgtggccc cgctctttcc tggagagcgt ctccttttct tccgctctta tgtcccatta 1320

aaaggtggat ttggaaaccc tgctatagat tgttcggtgc ctcaggagtg ggtccaacat 1380
ttctatcagg aatctgcccc ttctctgggg gatgtggcct tagttaggta cgtcaaccca 1440
gacaccgggc gcgtcctttt cgaggccaaa ctccacaaag gtgggttcct gactgtgtct 1500
agtactagca cagggcctgt tgtggttcca gccaatggct atttcaaatt tgattcctgg 1560
gttaatcaat tttactctct tgcccccatg ggaactggaa atgggcgtag aagggttcag 1620
taa 1623

<210> 19
<211> 1608
<212> DNA
<213> Hu/NLV/Chitta 1876/1996/JP

<400> 19
atgaagatgg cgtcgaatga cgccgctcca tctaatgatg gtgcagccgg tcttgtacca 60
gaggctaaca atgagaccat ggcacttgaa ccggtggctg gggcttcaat agccgcccca 120
ctcaccggcc aaaacaatat tatagacccc tggattagat taaattttgt gcaggctccc 180
aatggagagt tcacggtttc accccgcaac tcacccgggg aagtcctatt aaatttggaa 240
ttaggccccg aactaaatcc atacctagca caccttttcta gaatgtataa tggttatgca 300
ggtggggttg aggtgcaagt actactggct gggaatgcgt tcacagctgg aaaattggtg 360
tttgccgcag ttccccctca ttttccatta gaaaacataa gccctggtca gataactatg 420
tttcctcatg taattattga tgttaggact ttagaaccag ttttgttgcc ccttcctgat 480
gttaggaata atttctttca ttataatcag cagaatgaac cgaggatgag actcgtagca 540
atgctttata ctcctcttag atctaatggt tctggtgatg atgtatttac tgtctcctgc 600
agggtgctta cccgaccttc ccctgatttt gattttaatt acttggtccc ccctaccctt 660
gaatctaaaa ctaaaccctt cacactccct atcttgacta taggggagtt aaccaactcc 720
aggttccctg tgcccataga tgagctctac accagcccca atgagagtct ggtggtgcaa 780
ccccagaacg ggagatgcgc gctagatggg gagctacagg gcacgactca gctcctcccc 840
acggcgattt gctcgttcag gggccggatc aatcaaaagg tgagtggaga aaaccatgtt 900
tggaatatgc aggtcaccaa catcaacggg acccctttg atccaacagg ggatgtcccg 960
gctcctctag gaaccccaga tttctctggc aagctctttg gtgtactaag ccagagagac 1020
catgataatg cctgtaggag tcatgatgca gtaattgcaa ccaactctgc caaattcact 1080
ccaaaattgg gcgctataca aattggcaca tgggaagaag acgatgtgca catcaaccaa 1140
cctactaagt ttactccagt tggcttgttt gaaaatgaag gtttcaacca gtggacactc 1200
cccaattatt ctggagcctt aacacttaat atggggttgg cccctcctgt ggcccccacc 1260
ttccctggtg aacaaattct tttctttaga tcccacattc ctcttaaagg aggtgtggcg 1320
gacccagtta ttgattgtct cttgcctcaa gagtggatcc aacatcttta ccaagagtcg 1380
gcccctttcac aatcagatgt agcattgatt aggtttacaa atccagacac aggacgtgtt 1440
ctatttgaag caaaattaca caggagtggt tacattacag tggccaatac tggtagcaga 1500
ccgattgtgg taccagctaa tggttacttc aggtttgata cttgggtcaa tcaattctat 1560
tctctcgccc ccatgggaac tggaaatggg cgtagaaggg ttcagtaa 1608

<210> 20
<211> 1629
<212> DNA

<213> Hu/NLV/Kashiwa 47/1997/JP

<400> 20
atgaagatgg cgtcgaatga cgccgctcca tcaaatgatg gtgcagctag tctcgtacca 60
gagggcatta atgagactat gccattggaa cccgttgctg gcgcatctat tgctgcccca 120
gtggcgggac aaaccaacat aattgacccc tggataagaa caaattttgt acaagccccc 180
aatggagagt ttacagtgtc accaagaaat tcccctggag aaattttatt aaatttagaa 240
ttaggaccag atctgaatcc ttatttggcc catctttcaa gaatgtacaa tggttatgct 300
ggaggtgttg aggtgcaagt gctccttgct gggaacgcgt tcacagcagg taagatattg 360
tttgcagcaa tcccacctaa ctttctcgta gatatgatta gcccagctca aattactatg 420
cttccccatt tgattgtaga tgttaggact ttggaaccta ttatgacacc cttgcctgat 480
gttaggaatg tgttctatca ttttaataat caacctcaac ctagaatgag gttagtggct 540
atgctctaca ccccattgag gtctaatggt tcaggagatg atgtcttcac tgtgtcttgt 600
agagtactaa ctaggccaac tcctgatttt gaatttattt acctggtgcc cccttctgta 660
gagtccaaaa ctaaaccatt cacactacca atattaacca tttctgaatt gaccaactcc 720
cggttcccca ttccaatcga gcaattgtat acggctccaa atgaaaccaa tgttgtccag 780
tgtcagaatg gcaggtgcac cttagatgga gagctccagg gcacaaccca gctgttatca 840
agtgcagttt gcttcttaca gggcaggact gtggctgata atggggataa ttgggaccaa 900
aatttgctcc agctgaccta tccaaatggt gcaagctatg accccactga tgaagtgcca 960
gcaccattgg gcactcagga ttttagtggg atgttgtatg gagtgttgac ccaggacaat 1020
gtgaatgtga gcacaggaga ggccaaaaat gctaagggaa tatacatatc caccactagt 1080
ggaaaattca ccccaaaaat tgggtcaatt ggattgcatt caataactga gcatgtgcac 1140
cccaaccaac agtcgcggtt cacccccgtc ggagtcgccg tggatgagaa cacccccttc 1200
cagcaatggg ttctgccaca ttatgcaggt agtctcgctc tcaacaccaa tttggcacct 1260
gctgttgccc cgactttccc tggtgagcaa ttgctgttct tcaggtcccg tgtcccatgt 1320
gttcaaggcc tacagggaca ggatgcgttc atagattgcc tcctgcccca agagtgggtg 1380
aatcattttt accaagaggc agcccccttcc caagcagacg ttgcccttat taggtatgtc 1440
aaccctgata ccggtcgcac gctgtttgaa gccaaattgc atagatcagg ttttattact 1500
gtgtcacata ctggtgctta ccctcttgta gtccccccaa atggtcattt caggtttgat 1560
tcttgggtta atcaatttta ctcactcgcc cccatgggaa ctggcaatgg gcgtagaaga 1620
attcagtaa 1629

<210> 21
<211> 1653
<212> DNA
<213> Hu/NLV/Mie 7k/1994/JP

<400> 21
atgaagatgg cgtcgaatga cgctgctcca tcgaatgatg gtgctgccaa cctcgtacca 60
gaggccaacg atgaggttat ggcacttgaa ccggtggtgg gagcctcaat tgcagctcct 120
gttgtcggcc agcaaaatat aattgacccc tggattagag aaaattttgt ccaagcacca 180
caaggtgagt tcactgtttc accaaggaat tcgcctggcg agatgctctt aaaccttgag 240
ttgggcccag aacttaatcc ctatttaagt catttgtccc gcatgtacaa cggatatgct 300
ggtggcatgc aggttcaggt ggtcctagct gggaatgcgt tcacagctgg gaaaatcatc 360
tttgccgccg tgccaccaca tttccctgta gaaaacatca gtgcagccca aataactatg 420

tgtccccatg tgattgttga tgtgagacaa cttgaaccag tgcttctgcc cctccctgat 480
ataaggaata ggttcttcca ctacaaccag gagaacaccc cccggatgag gcttgtagcc 540
atgctctata cacctctaag ggctaactct ggtgaggatg tattcactgt gtcctgcagg 600
gttctgactc gccccgcccc agattttgag ttcacatttt tagttccacc aactgttgaa 660
tcaaaaacaa aacccttttac tttacctatc ttgactcttg gcgagttgtc taattctcgc 720
tttccggctg ctatagatat gctttatact gaccctaatg aatcaatagt tgtacaaccc 780
caaaatggta ggtgcaccct tgatggtaca ttgcaaggca caacacaatt ggttcccaca 840
cagatctgtg cttttagagg caccctgatc agccagaccg cgagagcggc cgattcaaca 900
gattcccccc agagagcccg taatcatcca ctgcacgtcc aagttaagaa cctagacggt 960
acacaatatg acccaacgga cgatatacct gcagtcctgg gggctattga cttcaaaggt 1020
acagtctttg gagtggctag tcagagggat gtttctggac aacaagaaca gggccactat 1080
gccacccgag cccatgaagc acacatcgac acaactgatc caaagtatgc acccaaatta 1140
ggcacaattc tcattaaatc tggttctgat gatttcaata caaaccagcc cattagattc 1200
actccggtgg gcatgggtga caacaattgg agacaatggg aattgcccga ctattctggc 1260
agattaacct taaatatgaa ccttgctcct gctgtttctc catctttccc tggtgaacga 1320
atccttttct tcaggtccat agtaccatca gccggaggct acgggtctgg ctacatagac 1380
tgtctcatac cccaggaatg ggtgcagcac ttttaccagg aagcagcacc ttcacaatct 1440
gctgttgcac tggttaggta tgtcaacccc gatactgggc gtaacatctt tgaggccaaa 1500
ctgcacagag aagggttcct caccgtggcc aactgtggaa acaatcctat tgtagtcccc 1560
cccaatggct atttcagatt tgaggcttgg ggtaatcagt tttatacact tgcccccatg 1620
ggatctggac aggggcgtag aagggcccag taa 1653

<210> 22
<211> 1626
<212> DNA
<213> Hu/NLV/Osaka 10-25/1999/JP

<400> 22
atgaagatgg cgtcgaatga cgcagctcca tctagtgatg gtgcagcagg cctcgtacca 60
gagatcaaca atgaggtcat gccccttgaa cccgtggctg gtgcatcgct ggcgacacca 120
gtcgtcgggc aacaaaatat aattgatccc tggataagaa ataattttgt gcaggctcct 180
gcaggtgagt ttactgtttc ccctaggaat tcccctggag aaattttgct tgatttggaa 240
ttgggaccag atttgaatcc ctacctagcc catctggccc gcatgtataa tgggcacgct 300
ggcggcatgg aagtgcaaat tgtgctggct gggaatgcgt tcacagcagg caaaatcata 360
tttgctgcca tccccccagg gttcccatat gaaaatttgt caccttctca aattacaatg 420
tgcccacatg ttataattga tgttaggcaa ttggagccat tccttttgcc tatgccagac 480
atttggaata atttcttcca ttataatcag ggcaatgatc caaaattgag gctagttgct 540
atgctctata ctcctttgag ggctaataat tctggtgatg atgtgttcac agtttcttgt 600
agggtgctca caaaaccttc acccgacttt gaattcacat ttctagttcc ccccacagtc 660
gagtctaaga ctaagcaatt cgctctgccc attctcaaaa tatcagagat gactaattca 720
agattcccag taccagtgga tgtgatgtac acggccagga acgagaacca ggtcgtccaa 780
ccacagaatg gcagggtcac actcgacggt gaactgttgg gcaccactcc cctgttggct 840
gttaacatct gtaaatttaa gggagaagtc atagccaaaa atggggacgt gagatcctat 900
agaatggata tggaaatcac taacactgat ggaacaccta ttgaccccac agaggacaca 960
cctggtccca ttggctcacc agattttcag ggcatacttt ttggcgttgc cagtcagcgc 1020

aataagaatg agcaaaaccc cgccacgagg gctcatgaag ccataattaa cactggtgga 1080
gaccatttat gcccccaaat tagctcaagt gaaatttatc tcacaagtcc caacattttg 1140
aggtgcacca acccacaacc tttaccccag tcggggttgc gggggacaat tctcatccgt 1200
tcagacaatg gacactgcca cgatatggtg ggcacctcac caacaacacc cacctggccc 1260
cagcagtggc gccgctgttc ccgggggagc aattgctgtt cttcaggtca cagataccca 1320
gttccggtgg tcatgaatcg cgttacatgg attgtcttgt cccacaagag tgggttcagc 1380
acttctacca ggaagctgcc acagctcaat ctgaggtggc ccctcataag attcatcaac 1440
ccagacactg gtagggtcct tttgaggct aggctacata agcaaggctt cataactgtg 1500
gctcataccg gtgacaaccc aattgtcatg ccaccaaatg ggtatttcag gtttgaagct 1560
tgggtcaatc agttttattc acttgccccc gtgggaactg ggaaagggcg tagaagggtc 1620
caataa 1626

<210>23
<211>21
<212>DNA
<213>Artificial Sequence

<400>23
aatgatgatg gcgtctaagg a 21

<210>24
<211>33
<212>DNA
<213>Artificial Sequence

<400>24
tttttttttt tttttttttt tttttttttt ttt 33

<210>25
<211>24
<212>DNA
<213>Artificial Sequence

<400>25
gccattatcg gcgcaracca agcc 24

<210>26
<211>20
<212>DNA
<213>Artificial Sequence

<400>26

tgacctcgga ttgtggacag 20

<210>27
<211>31
<212>DNA
<213>Artificial Sequence

<400>27
gcgaattctt atctacggac accaagccta c 31

<210>28
<211>20
<212>DNA
<213>Artificial Sequence

<400>28
gtgaatgaag atggcgtcga 20

<210>29
<211>23
<212>DNA
<213>Artificial Sequence

<400>29
ccattataat gcacgcctgc gcc 23

<210>30
<211>22
<212>DNA
<213>Artificial Sequence

<400>30
ttgtgaatga agatggcgtc ga 22

<210>31
<211>24
<212>DNA
<213>Artificial Sequence

<400>31
aattattgaa tccttctacg cccg 24

<210>32
<211>28
<212>DNA
<213>Artificial Sequence

<400>32
aattactgaa cccttctacg cccatttc 28

<210>33
<211>23
<212>DNA
<213>Artificial Sequence

<400>33
ccataactga acccttctac gcc 23

<210>34
<211>24
<212>DNA
<213>Artificial Sequence

<400>34
atgaagatgg cgtcgaatga cg 22